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Dr GUY RICHARD BISBY
1889–1958

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OBITUARY

DR GUY RICHARD BISBY

(1889–1958)

Dr G. R. Bisby, a warm friend of many members of the Society, died at his home in Staines, Middlesex, on 3 September 1958, after an illness lasting nearly four years.

Born a United States citizen at Brookings, South Dakota, on 17 August 1889, Guy Richard Bisby spent the greater part of his life on the prairies. From high school he put himself through college, working at one time as hospital orderly and later as laboratory assistant. He studied first at South Dakota State College at Brookings, taking his B.Sc. in 1912, and working there a further year before proceeding to Brooklyn Botanic Garden, New York. This move launched him on the first phase of his career—that of plant pathologist. Studying at Columbia University for his M.A. degree in 1914 and 1915, he worked on potato diseases during the summer months, first with W. A. Orton, J. E. Melhus and William Stuart at the Bureau of Plant Industry, Washington, and later as consulting pathologist to the American Potato Corporation at Presque Isle, Maine. In 1915 he was appointed Assistant in Botany at the Indiana Agricultural Experiment Station, Lafayette. In 1916 he married Helen Hitchcock, and they went west to Minnesota for an appointment at the University where he was soon promoted to Assistant Professor of Plant Pathology in the lively company of E. C. Stakman and his colleagues. During four happy years there he gained the degree of Ph.D. (Minnesota). In 1920 Professor A. H. R. Buller persuaded him to cross the frontier to become Professor of Plant Pathology in the University of Manitoba, Winnipeg, Canada. Almost at the same time he suffered a grievous loss when his wife became fatally ill while she was on a journey east.

In Winnipeg Bisby developed his teaching classes and continued to publish research on plant pathology. A year's leave of absence to England in 1921–22 gave him a taste of what was then the Imperial Bureau of Mycology, stimulated his interest in systematic mycology, and launched him on the second phase of his career. Returning to Winnipeg, he and Buller started assiduous collecting in mycologically unknown territory: the poplar groves around the university campus on the bank of the Red River; the Canadian prairies; and the lake and rock country north and east of Lake Winnipeg. This work bore fruit in a book by Bisby, Buller and the veteran Canadian mycologist John Dearness, *The Fungi of Manitoba* (1929), enlarged in a later edition to include Saskatchewan. In those years at Winnipeg Bisby was an essential component of the hive of mycological activity, including the brilliant team at the Dominion Rust Research Laboratory and A. M. Davidson at the Medical School, which

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developed around Buller at the university. The close friendship and association between Bisby and Buller persisted, and during Professor Buller's last illness in 1944 Bisby was able to set his friend's mind at rest by voluntarily undertaking to edit and prepare for press the final volume of Buller's *Researches on Fungi*, later published by the Royal Society of Canada.

In Winnipeg Dr Bisby's unobtrusive hospitality helped newcomers to feel at home in the West, and foreign students in particular received most practical help from him. His recreations made him friends. Music was a lifelong interest; he played the clarinet from Minneapolis days, he conducted a small orchestra on the Winnipeg campus, and in later years he played again in Richmond. Chess was another absorbing interest throughout his life.

In 1936 he married Miss Berenice Butler, the English artist and illustrator of children's books. They came to live in England in 1937 when he was attracted to a post at the Commonwealth Mycological Institute. Here his ability to make decisions quickly and his impatience always to get on to the next task earned him the gratitude of a generation of mycologists who referred specimens to him for identification and could rely on a prompt and cheerful reply. Bisby's major publications from the Institute include: *The Fungi of India* (1931) with Butler which he compiled when, on sabbatical leave, he deputized for Mr E. W. Mason for six months at the Imperial Mycological Institute, *The Fungi of Ceylon* (1950) with Petch, and his sensible little guide to the *Taxonomy and Nomenclature of Fungi*. Collaboration with G. C. Ainsworth produced the several editions of the invaluable *Dictionary of the Fungi*. Bisby also published substantial papers on the Hysteriaceae, on the genera *Cladosporium*, *Trichoderma* and *Stachybotrys*, and numerous other works.

Bisby joined the British Mycological Society in 1921 and served it generously but unobtrusively. He seldom came to meetings and avoided committees whenever possible. He was modest about his great ability and retiring in disposition, but the best of company when deliberately dug out; his work and recreations were too efficiently organized to give him time for administrative tasks. His notable contributions to the Society were scientific, and include his share in compiling the lists of British Fungi published in the *Transactions*, especially *Pyrenomycetes* with Mr E. W. Mason, *Hyphomycetes* with Miss E. M. Wakefield, and *Rusts* with Dr Malcolm Wilson. For the Society's Jubilee he prepared the 50-year Index to Volumes 1–30 of the *Transactions*. (This went to press in longhand, the printers agreeing that Bisby's handwriting was more legible than typescript.)

Residence in England developed new interests. Weekends and holidays found the Bisbys avidly exploring the countryside with its landscape, villages and prehistoric sites. A small garden beside the Institute remained a source of satisfaction, producing squashes and sweet corn, after the wartime need for potatoes had gone.

Dr Bisby retired from the Commonwealth Mycological Institute in 1954, and a few days later was stricken with a tedious illness which kept him almost confined to bed until his death. He received great encouragement during this period when in 1956 his contribution to systematic mycology was recognized by the award of the University of Minnesota Gold Medal for outstanding achievement.

The Society is the poorer for the loss of a kind and often gay companion, an original, honest and orderly mind, a man whom we never got to know well enough.

P. H. GREGORY.

(Other notices have appeared in: *Nature, Lond.*, **182**, 987, 1958, and *Taxon*, **8**, 2, 1959, by G. C. Ainsworth.)

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THE CYTOLOGY OF *GLOEOTINIA TEMULENTA* (BLIND SEED DISEASE OF RYE-GRASS)

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(With Plates 7 and 8 and 3 Text-figures)

A study has been made of nuclear organization and reproduction during the life-cycle of *Gloeotinia temulenta.* All cells of the vegetative hyphae are uninucleate and heterokaryons cannot, therefore, occur; the only sources of genetic variation are thus mutation and sexual recombination. The chromosome number, $n = 15$, has been determined at both mitosis and meiosis. The mitotic chromosomes range in size from 0·25 μ to just under 1 μ. Most, if not all, of the chromosomes are highly heterochromatic. Prominent 'prochromosomes' are found in resting nuclei which approximate in size and number to the mitotic chromosomes. At both mitosis and meiosis 'heterochromatic association' is a prominent feature.

Nucleolar activity (as indicated by variation in size) has been examined in relation to both cytoplasmic protein synthesis and nuclear reproduction. Nucleolar activity is positively correlated with cytoplasmic basophilia and is greatest in cells which must be presumed to be actively engaged in protein synthesis, viz. young vegetative hyphae and young asci. The apparent absence of the nucleolus from microconidiophores where the nucleus undergoes repeated division indicates that it is not essential for nuclear reproduction. Microconidia in *G. temulenta* (as in other members of the Sclerotiniaceae) do not germinate and produce a vegetative mycelium; their function is purely sexual. Their inability to germinate is attributed to the very low concentration of ribose nucleic acid (RNA) in the cytoplasm which results from an elaborate pattern of cytological and cytochemical differentiation in the microconidiophores and associated cells.

The ascogonium in *G. temulenta* consists apparently of a coiled hypha embedded in a small stroma. In stromata fertilized by compatible microconidia, hyphae with binucleate cells are produced and these are interpreted as young ascogenous hyphae which subsequently grow up the centre of the apothecium and proliferate in the subhymenium to yield the asci. Nuclear behaviour in the ascus shows no peculiarities. Meiosis is followed by a mitotic division to yield eight nuclei; a single ascospore is organized around each nucleus.

Introduction

In an earlier paper (Griffiths, 1958) an account was given of the genetical aspects of sexual reproduction and variation in *Gloeotinia temulenta*, the causal organism of blind seed disease of rye-grass. It was shown that the fungus is obligately heterothallic with a bipolar mating system and that sexual reproduction involves fertilization of small stromata (presumed to contain ascogonia) by microconidia. While these genetical studies were in progress it was considered important to examine certain features of the cytological organization of the fungus and observations on these aspects are reported here.

In the Sclerotiniaceae sexual development and cytology have hitherto been studied only in *Sclerotinia*. Several species of *Sclerotinia* are known to be heterothallic: *S. gladioli* (Mass.) Drayt. (Drayton, 1934); *S. convoluta* Whetz. & Drayt. (Drayton, 1937); representatives of the *Botrytis cinerea* Pers. group (Groves and Drayton, 1939). The cytology of these species would clearly have a particular significance for the present studies, but unfortunately none has been investigated in great detail. However, in *S. gladioli*, Drayton (1934) made important observations on nuclear behaviour in 'receptive bodies' and 'apothecial fundaments'. He was able to demonstrate that ascogonial coils in spermatized 'receptive bodies' showed pairing of the nuclei and that these paired nuclei passed out into the ascogenous hyphae. His observations did not, however, extend to the development of the ascus nor were any observations made on nuclear reproduction.

In *Sclerotinia trifoliorum* Erikss. (homothallic) and *S. fructicola* (Wint.) Rehm. (mating system undetermined), detailed cytological studies have been made. In *S. trifoliorum*, Bjorling (1942) was able to show that ascogonia are lacking and in *S. fructicola*, Heuberger (1934) was unable to detect any structures which could be identified as ascogonia. Microconidia are produced in both species but there is no evidence that they are functional. The development of asci and nuclear behaviour in the ascus showed no peculiarities in either species and followed the pattern commonly reported in Ascomycetes. The chromosome numbers were given as $n = 8$ in *S. trifoliorum* and $n = 4$ in *S. fructicola*, but in neither case was convincing photographic evidence provided.

The formation of microconidia is a striking feature of many members of *Sclerotinia* and of *G. temulenta*. Even though wide variations occur in the form of asexual conidia the microconidia are remarkably uniform. In the Sclerotiniaceae they lack the capacity to germinate and reproduce the vegetative phase of the fungus. They are thus sharply differentiated from propagative spores (asexual conidia and ascospores) and it might be supposed that they would show differences in either structure or development. It is surprising, therefore, that little interest has been taken in their cytology. Heuberger (1934) claimed that in *S. fructicola* the single nucleus present in the microconidium was crescent- or comma-shaped, and similar observations were made by Drayton (1934) for *S. gladioli* and by Beauverie & Guillermond (1903) for *Botrytis cinerea*. However, in no case were the processes leading to spore development studied in detail. In *G. temulenta* the microconidia proved surprisingly favourable for cytological study and consequently particular attention was paid to them.

MATERIALS AND METHODS

Materials

(i) *Gloeotinia temulenta*. Cultures on either complete agar medium (Pontecorvo, 1949) or in a solution of the nutrients present in complete medium were used to provide material of hyphae and conidia. Cultures which had been grown for 10–14 days at 20° C. were generally used. Microconidia were not usually produced on complete medium but were occasionally abundant on a synthetic medium containing, in addition to basal nutrients (Pontecorvo, 1949), biotin, aneurin and casein hydrolysate. Observations were made on microconidial sporodochia obtained from such cultures. Apothecia were readily obtained from naturally infected seeds by cold treatment (Griffiths, 1958). The ascogonial stromata studied were observed on only one occasion on artificially infected seed.

(ii) *Neurospora crassa.* In order to provide a comparison with *G. temulenta*, a study was also made of the microconidiophores and microconidia of *N. crassa*. The strain used was Y8743 (Barratt & Garnjobst, 1949) which produces abundant microconidia. Cultures were grown on Difco cornmeal agar for 14–21 days at 20° C.

General cytological methods

Any technique for the critical study of the small nuclei and chromosomes of fungi must provide for (*a*) satisfactory fixation, (*b*) staining to give high contrast between nuclear and other structures and the surrounding cytoplasm, (*c*) a manipulative or other procedure for dispersing the stained material so that the small structures present may be resolved. These three requirements can be fulfilled only by what are generally termed 'squash' or 'smear' techniques. These techniques were readily applicable to the study of the hyphae, conidia, microconidia and asci; they were not, however, suitable for study of the development of the apothecium and for this work microtome sections had to be employed.

Squash techniques

Fixation in acetic-alcohol (3 ethyl alcohol; 1 glacial acetic acid) gave excellent results. Other fixatives tried included various combinations of acetic, propionic and butyric acids, methyl, ethyl and butyl alcohols, formalin and chloroform. Many of these were satisfactory but none gave results superior to acetic-alcohol. The use of a premordant (Thomas, 1940) was not found to improve the subsequent staining with aceto-carmine. Aceto-carmine (with subsequent addition of iron, as ferric acetate) was the only stain which gave satisfactory results; staining with acetic-orcein and aceto-lacmoid was very poor.

The problem of separating the chromosomes at both mitosis and meiosis proved extremely difficult. It soon became clear that critical observations would be possible only if the cell contents could be forced out of the cell and adequately dispersed. Fungal cell walls are, however, surprisingly tough and do not break easily. Attempts were made to soften the wall by hydrolysis in hydrochloric acid (HCl), treatment with snail cytase (Faberge, 1945; Singleton, 1953) and treatment with a mixture of ammonium oxalate and hydrogen peroxide (Ford, unpubl.; cited by Lewis, 1956), but all proved ineffective. Consequently a manipulative technique was developed which, though not completely satisfactory, did give some useful results. After staining the material and mounting it under a coverslip, sharp tapping was used to disperse the material evenly and to fracture as many cells as possible. The slide was then heated and, while still warm, firm pressure was applied to the coverslip. By this means the contents of some of the cells could be spread out. The success of the method varied with the nature of the material; hyphae were particularly recalcitrant and asci were only a little better, but microconidiophores responded reasonably well.

The staining of hyphae was generally poor and this was at first attributed to the presence of agar round the hyphae. However, material from liquid culture did not give significantly superior results, and it was concluded that the presence of large quantities of basophilic material in the cytoplasm was responsible for the poor results. Hydrolysis in N-HCl for 15–20 min. at 60° C. produced a much clearer cytoplasm and, though the subsequent staining of nuclei and chromosomes was less intense, observations could be made more easily.

Sectioning technique

Material was fixed by immersion in Carnoy for 1 min. followed by Randolf's fixative for 24 hr. (Darlington & La Cour, 1947). The fixed material was embedded following dehydration in tertiary butyl alcohol (Johansen, 1940). Sections were cut at $5\,\mu$ and stained in crystal violet, using the chromic modification for deeper staining (Darlington & La Cour, 1947).

Cyto-chemical techniques

In studying the cytology of the microconidiophores an attempt was made to determine the distribution and relative abundance of both deoxyribose (DNA) and ribose (RNA) nucleic acids. The following methods were employed.

(i) *Feulgen reaction.* The significance of this reaction for studies on the distribution of DNA has recently been reviewed by Swift (1955), and his procedure has been adapted for the present study. Fixed material was gently macerated in acetic-alcohol to produce a suspension consisting of small groups of microconidiophores. Subsequent handling of the material was facilitated by the use of a small centrifuge. The material was hydrolysed in N-HCl, the optimum hydrolysis time of 10 min. being determined by removing samples at intervals of 2 min. After hydrolysis the material was washed in water and transferred to Feulgen reagent (pH 2); very acid reagent ($pH < 1{\cdot}5$) was unsatisfactory. The time of staining produced no significant variation over the range 1–48 hr. After staining, the cells were washed in an hydrochloric acid-potassium metabisulphite solution (Swift, 1955) and then transferred to water. A drop of the suspension was mounted under a coverslip. Gentle tapping served to distribute the cells, and, after applying firm pressure, the coverslip was floated off in 10% acetic acid. Following dehydration in a water-alcohol series the coverslip and slide were reunited with Euparol.

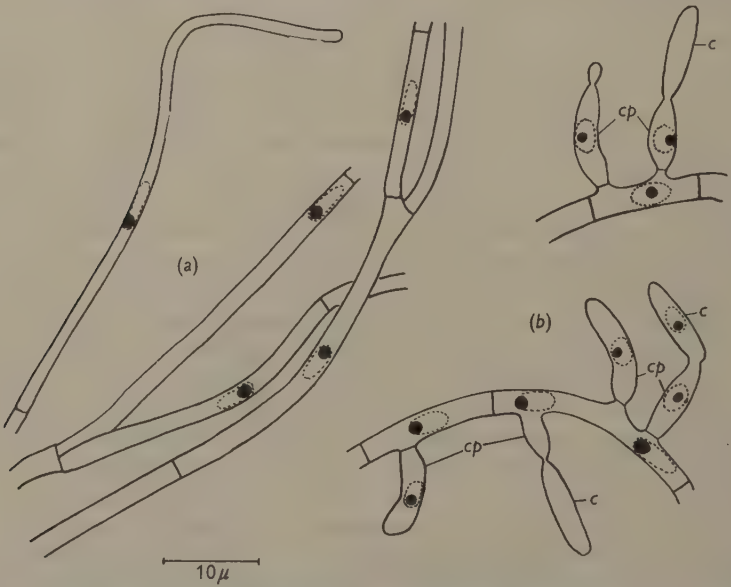
(ii) *Methyl green and toluidine blue.* Korson (1951) has developed this particular stain combination and claims that it gives quantitative staining of DNA (green) and RNA (blue). Korson's procedure was adapted for the present material. A suspension of cells in acetic alcohol was prepared and mounted under a coverslip. Firm pressure was applied to the coverslip which was then floated off in 10% acetic acid. With this method, most cells adhered to the coverslip, which was then passed through the various staining solutions. Differentiation was in tertiary butyl alcohol and after clearing in xylol the coverslip was affixed to a clean slide with balsam.

In order to make a critical study of the distribution and relative amounts of RNA, it was necessary to stain control material from which RNA had been removed by incubation with ribonuclease. Coverslips with adhering material were washed thoroughly with water and transferred to a solution of ribonuclease (1 mg./ml.) at pH 6·5 and incubated for 2 hr. at 30° C. (the ribonuclease was obtained from L. Light and Co.).

OBSERVATIONS

Hyphae and conidia

The hyphae of *G. temulenta* are rarely more than 2–3 μ in diam. The individual cells are delimited by septa and vary considerably in length; apical cells are often 60–70 μ long but intercalary ones, and particularly those associated with conidiophores, may be no more than 10 μ.



Text-fig. 1. Distribution of nuclei in hyphae, conidiophores and conidia. (*a*) hyphae; both terminal and interstitial cells are uninucleate; (*b*) conidiophores (*cp*) and conidia (*c*).

All cells are regularly uninucleate (Text-fig. 1*a*). Conidiophores arise laterally on the hyphae and are short barrel-shaped structures, 2–3 μ wide and 5–15 μ long. They may be continuous with the hyphal cell, from which they developed, or cut off by a septum (Text-fig. 1*b*), in which case both the conidiophore and the subtending hyphal cell contain a single nucleus; where, however, the conidiophore is continuous with the hyphal cell a single nucleus (usually situated at the base of the conidiophore) serves both structures. The conidia (12–19 × 3–4 μ) are budded out from the apex of the conidiophore and attain almost their full size before nuclear division occurs in the conidiophore. A single daughter nucleus passes into the conidium.

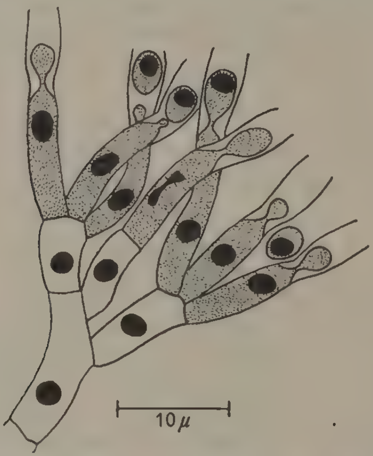
The vegetative nucleus, which is large in relation to the hypha, is oval in shape and measures 3–5 × 2 μ. It consists of a prominent nucleolus, which may be as much as 2 μ in diam., and numerous darkly staining granules (Pl. 7, figs. 2, 3). These could not be counted accurately but appeared to number between six and twelve. Many attempts were made to study nuclear reproduction in hyphae and conidiophores but the nature of the material was such that satisfactory staining could not be obtained. Further, it proved virtually impossible to achieve adequate spreading of the chromosomes. As far as could be observed, however, nuclear reproduction was essentially similar to that found in microconidiophores, where much more detailed observations were possible.

Microconidia

Microconidia are produced naturally on infected seeds which have either overwintered or been exposed, under moist conditions, to low temperatures. They were also produced abundantly in agar cultures on certain synthetic media. The microconidia are budded out from specialized cells, the microconidiophores, and these are organized into compact cushion-like structures—sporodochia. The sporodochia are pale pink and, when they occur on seeds, are formed either on the surface of the pales or between the pales and the caryopsis.

The sporodochium consists of a core of closely septate, branching hyphae; the terminal cells of each branch bear 1–4 microconidiophores which constitute the surface layer of the sporodochium (Text-fig. 2). Each microconidiophore buds off conidia at its apex. The first microconidium to be formed is produced within the tip of the microconidiophore and is released by its rupture. This gives the microconidiophore its characteristic apex consisting of a short open tube at the base of which is the 'growing point', from which successive microconidia are budded out. The microconidiophores are about 3 μ in diam. and their length (including the apical tube, which varies from 3 to 6 μ long) varies from 15 to 20 μ. The microconidia are oval and measure approximately 2 × 3 μ.

The cytoplasm of the microconidiophores is remarkably free from granules and is not highly basophilic. These features enabled detailed observations to be made of nuclear behaviour. The resting nucleus has the granular structure found in the nuclei of vegetative cells but, unlike the vegetative nucleus, regularly lacks a nucleolus (Pl. 7, fig. 1). The prophase of mitosis is not clearly defined and the only change from the resting stage to metaphase is greater stainability and the emergence of more clearly defined granules—the chromosomes (Pl. 7, fig. 6). Although the chromosomes are highly aggregated at metaphase it was possible to study them in some detail since the microconidiophore ruptured fairly easily and the contents could be well dispersed. A number of metaphase plates in which the chromosomes were well spread were found, and one of these is shown in Pl. 7, fig. 4. The mitotic chromosomes, even after they have been subjected to severe pressure, are minute; the largest measure less than 1 μ and the smallest are no more than 0·25 μ, which approaches the limit of resolution; it is obviously impossible to study such small structures in great detail. However, it was regularly noted that the chromosomes, particularly the large ones, tended to stick together, being united by strands of stainable material. This is particularly well illustrated by the four large chromosomes shown in Pl. 7, fig. 4.



Text-fig. 2. Microconidiophores and microconidia. Nuclei are shown black; cytoplasm of the microconidiophores is stippled and of the subtending cells unstippled.

Anaphase (Pl. 7, fig. 7) was rarely observed and presumably proceeds rapidly. At this stage the chromosomes are not clearly defined and it is as though a sticky mass were being pulled apart. The two daughter nuclei are at first very condensed and show no sign of granular structure. While nuclear reproduction is occurring the development of the microconidium 'bud' is completed. One daughter nucleus migrates into the 'bud' via a narrow connecting pore (Pl. 7, figs. 9, 10) and the newly formed microconidium is then cut off from the microconidiophore. Its nucleus is highly condensed and shows no sign of structure. Often it is pressed closely against the spore wall and this would appear to account for the crescent- or comma-shaped appearance noted by earlier workers on similar fungi.

Each microconidiophore is active for only a limited time; a stage is reached when the nucleus in the microconidiophore ceases to divide and finally migrates into the last microconidium to be formed (Pl. 7, figs. 11, 12). This process appears to be correlated with a decline in basophilia (Pl. 7, fig. 13) of the cytoplasm so that when the last microconidium is formed the cytoplasm is completely unstained. High basophilia is often considered to be an indication of high concentration of nucleic acid and these observations therefore suggested that microconidia production might, in some way, be linked with the nucleic acid status of the microconidiophore. In order to study this point more critically, cytochemical tests were carried out to determine the distribution and relative amounts of both DNA and RNA respectively.

(i) *Feulgen reaction.* This proved difficult to perform on this material and positive results were obtained only under the narrow range of conditions described earlier. Even so only a weak reaction was observed, and that only in the nuclei which stained very faintly pink.

(ii) *Methyl green–toluidine blue.* Teased preparations of microconidial sporodochia, which contained three classes of cells (microconidia, microconidiophores and their subtending hyphal cells) showed the following reactions (see also Pl. 7, fig. 14). (*a*) Microconidia: nucleus green; very faint blue staining in the cytoplasm. (*b*) Microconidiophores: nucleus green; cytoplasm of active cells uniform pale blue; non-nucleated cells colourless. (*c*) Subtending cells: nucleus green; cytoplasmic staining varying from deep blue to colourless. When the cytoplasm of the subtending cells was colourless, the microconidiophores attached to them were either unstained and non-nucleated or showed only very faint blue staining in the cytoplasm (Pl. 7, fig. 15). Vacuoles which stained reddish were frequently present in the cytoplasm (toluidine blue shows metachromastasy and is reputed to stain mucins reddish).

Material which had been previously treated with ribonuclease to remove RNA showed after staining the following reactions. (*a*) Microconidia: nucleus green; colourless cytoplasm. (*b*) Microconidiophores: nucleus green; cytoplasm of nucleated cells showed a faint greenish tinge but no blue; non-nucleated cells had colourless cytoplasm. (*c*) Subtending cells: nucleus green; cytoplasm with faint greenish tinge but no blue. A faint pink was associated with vacuoles in some of the cells.

Microconidia of Neurospora crassa

In species of *Neurospora* and in the related *Gelasinospora calospora* (Sloan & Wilson, 1958), the microconidia differ from those of *G. temulenta* in that, although they are uninucleate and of comparable size, they are capable of germinating to produce a vegetative mycelium. Cytologically, this difference in behaviour is reflected in different patterns of both cellular and cytochemical organization. In *N. crassa* (Pl. 7, figs. 16, 17) the hyphae bearing the microconidiophores contain little RNA whereas the microconidiophores and the microconidia which develop from them are heavily charged with RNA.

Ascogonia

Stromata from which apothecia developed were first observed on seeds which had been artificially infected with *G. temulenta*. Stromata on seeds which had been originally infected by a single strain of the fungus failed to develop apothecia until they had been fertilized by microconidia from a compatible strain (Griffiths, 1958). These stromata, like the microconidial sporodochia, are formed both superficially, i.e. on the surface of the pales, and between the pales and the caryopsis. They are roughly spherical to pear-shaped and are invested with fine white hyphae. They generally occur in small groups and are united at the base by loosely woven hyphae.

Sections through unfertilized stromata showed, at the centre of each, a coiled hypha which was differentiated from the rest of the stroma by its larger cells (Pl. 8, fig. 1), which appeared to be regularly uninucleate. These coiled hyphae bore a close resemblance to the ascogonia described in *Sclerotinia gladioli* (Drayton, 1934) and also to ascogonia in other discomycetes such as *Higginsia hiemalis* (Backus, 1934). In these two species the ascogonium is connected to a long hypha, the trichogyne, which receives the microconidia. The ascogonial stromata of *G. temulenta* are invested with numerous hair-like hyphae, but it was not possible to trace a connexion between any of these and the ascogonium.

An attempt was made to study the further development of the ascogonia in stromata from seeds which had been inoculated with microconidia. Drayton (1934) found in *S. gladioli* that the ascogonial cells became binucleate after fertilization with microconidia. The initiation of a binucleate condition was not observed in the ascogonium in *G. temulenta* but hyphae with binucleate cells were detected at the centre of the ascogonial stromata. Binucleate cells were never observed in unfertilized stromata.

In young apothecia, in which the stalk had not elongated, deeply stained hyphae were regularly observed occupying a central position in the young apothecium (Pl. 8, fig. 2). Again, as far as could be determined, the cells of these hyphae also were binucleate. In apothecia approaching maturity young ascogenous hyphae, showing highly characteristic hook formation, could be detected in the subhymenium of the apothecium. At this stage no darkly staining hyphae could be found in the stalk of the apothecium and ascogenous hyphae in the subhymenium could be traced only to unstained hyphae, which were not clearly differentiated from the other hyphae comprising the stalk.

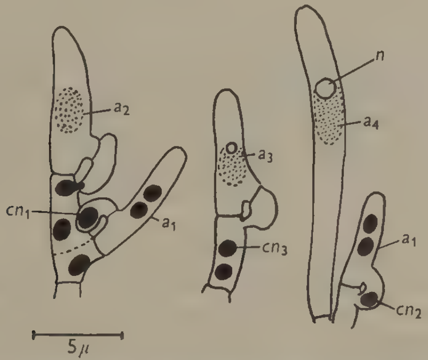
Development of the ascus

The development of the asci was studied in preparations made by teasing the hymenium of the apothecium and staining in aceto-carmine. The ascogenous hyphae produce asci by the process of proliferation common to most ascomycetes.

The nuclear behaviour associated with this stage of development is shown in Text-fig. 3 and in Pl. 8, figs. 3–5. In the young binucleate ascus, both nuclei are highly condensed and neither possesses a nucleolus. Nuclear fusion occurs at an early stage and is completed by the time the binucleate condition has been restored in the cell below the ascus. The fusion nucleus is at first highly condensed but rapidly becomes more diffuse and this change is accompanied by the development of the nucleolus. No further changes in the structure and behaviour of the nucleus could be detected until the onset of meiosis.

Meiosis

Owing to the minute size of the chromosomes it was not possible to follow in detail the changes in nuclear organization which precede metaphase I. On some occasions, however, stages were observed which could be interpreted as late prophase or diakinesis. Metaphase I was clearly marked by the appearance of deeply staining chromosomes and the disappearance of the nucleolus. As in mitosis, the chromosomes were invariably clumped together and detailed observations could only be made on asci which had been severely pressed to disperse the contents. In such preparations bivalent structure could be detected and, in all the cells which were analysed in detail, fifteen bivalents were present. One such cell is shown in Pl. 8, fig. 6. No precise estimate of chiasma frequency could be made. Ring bivalents, however, appeared to be most common, with rods occurring only rarely. Thus at metaphase the mean chiasma frequency would seem to be just under two. As in mitosis, considerable variation was observed in size of bivalents, and again there was a tendency for bivalents to stick together; even when separated under pressure, they often showed association by strands of stainable material.



Text-fig. 3. Stages in ascus development (see also Pl. 8, figs. 3–5). a_1, binucleate ascus; a_2, fusion nucleus in ascus (no nucleolus); a_3, early stage in development of the nucleolus; a_4, prophase nucleus; cn_1, single nucleus in terminal cell of crozier; cn_2, nucleus in terminal cell passing into cell below ascus; cn_3, binucleate condition established in cell below ascus.

Anaphase I was rarely observed and no preparations in which the chromosomes were well spread were obtained. However, in several cases it could be seen that the number of chromosomes passing to each pole was between ten and fifteen.

Following anaphase I, no interphase was observed and the chromosomes passed immediately to metaphase II. Again it was possible to demonstrate fifteen chromosomes (Pl. 8, fig. 8). Comparison with metaphase I shows that these chromosomes were approximately half the size of the bivalents and bore a close similarity in size to the mitotic chromosomes.

Division of the chromosomes at metaphase II leads to the production of the four-nucleate stage. The four nuclei, which are initially small and highly condensed, do not divide immediately; they enter an interphase condition and become so diffuse that they are barely distinguishable from the surrounding cytoplasm (Pl. 8, fig. 10). The chromosomes at this stage appear to be very elongated and resemble the prophase condition. They condense again to metaphase III (Pl. 8, fig. 11). The final mitosis is a sticky one and no detailed observation of the chromosomes was possible. Eight condensed nuclei are formed (Pl. 8, fig. 12) and these subsequently become very diffuse (Pl. 8, fig. 13).

The commencement of spore formation follows rapidly the appearance of the eight-nucleate stage. As these nuclei become diffuse, the cytoplasm surrounding them becomes organized into a spindle with the nucleus at the centre (Pl. 8, fig. 13). The spindle-shaped areas stain more deeply and there are indications of fibrils extending from the nucleus to the poles of the spindle. Finally a wall is organized over the surface of the spindle-shaped areas and the uninucleate ascospores are thus cut out from the surrounding cytoplasm. This pattern of behaviour has been generally found to occur during spore formation in ascomycetes and in exceptionally favourable material (powdery mildews, Harper, 1905; *Neurospora crassa*, Singleton, 1953) it can be shown that the organization is initiated by centrioles; these structures could not, however, be identified in *G. temulenta*.

Discussion

Cytological observations on *Gloeotinia temulenta* have been concerned with three levels of organization within the cell; first, with the numbers of nuclei present at various stages of development; secondly, with the structure of the nucleus and the behaviour of its component parts; and thirdly, with the role of nucleic acids in the differentiation of the microconidia.

Nuclear number

It has been shown that, with the exception of the cells in ascogenous hyphae, all cells in *G. temulenta* are strictly uninucleate. Heterokaryons cannot therefore be formed and consequently genetic changes can only arise through mutation and sexual recombination. The essential features of sexual reproduction have already been established (Griffiths, 1958); the fungus is obligately heterothallic and the sexual organs consist of ascogonial stromata and microconidia. Apothecia develop from ascogonial stromata only when these are fertilized by microconidia from a compatible strain.

Cytological study has shown that each ascogonial stroma contains a coiled hypha which in general appearance closely resembles the ascogonia found in related ascomycetes (*Higginsia hiemalis*, Backus, 1934; *Sclerotinia gladioli*, Drayton 1934). A similar function has therefore been inferred for the coiled hypha in *G. temulenta*. Further, following application of compatible microconidia, binucleate cells were seen in the centre of the ascogonial stroma and there can be little doubt that these belong to the ascogenous hyphae which subsequently grow up in the centre of the developing apothecium, emerging in the subhymenium to produce the asci. Since segregation has invariably been found to occur in apothecia of *G. temulenta* (Griffiths, 1958), it is evident that the pairs of nuclei present in the cells of the ascogenous hyphae must be genetically different, one having been contributed by the ascogonial parent and the other by the microconidium. Clearly the microconidial nuclei must pass from the exterior of the ascogonial stroma to the interior, where the binucleate cells were observed. In *H. hiemalis*, *S. gladioli* and in many other ascomycetes this is accomplished by means of a trichogyne or specialized hypha connected to the ascogonium, but this structure could not be detected in *G. temulenta.*

Clearly, more information is needed to provide a complete picture of the cytological processes involved in sexual reproduction. However, it is difficult to see how far further observations would enhance an understanding of the genetical behaviour of the organism. Their main value would appear to lie in providing further information about nuclear behaviour in ascogenous hyphae—an aspect where considerable diversity of opinion still exists (see, for example, Wilson, 1952). For such fundamental studies particularly favourable material is needed and it is significant that even in *Neurospora*, which has been fully investigated, certain aspects of nuclear behaviour can still only be inferred from genetical data (Catcheside, 1951).

Nuclear organization and behaviour

The resting nuclei in fungi have in general received little attention and there appears to be only one paper dealing exclusively with this aspect, that of Pinto-Lopes (1949). He has pointed out that fungal nuclei do not differ in any fundamental way from the nuclei of higher organisms and that the diverse forms which have been described may be readily accommodated within the classification devised by Serra (1947) for higher plants. Serra distinguished only two basic types of nuclei—*disperse* and *thread*—but these in fungi may present diverse forms, since each may exist in either an expanded or a non-expanded form. In fungi, the most common type is the disperse nucleus characterized by prominent chromocentres and this is the type found in *G. temulenta.* Pinto-Lopes (1949) showed great caution in the interpretation of the chromocentres but it would seem evident that they are in fact heterochromatic regions of chromosomes. Heterochromatin, in cytological terms, is chromosomal material which is allocyclic, i.e. undergoes differential condensation or heteropycnosis (Barigozzi, 1949), and the appearance of the resting nucleus in *G. temulenta* is such as would be expected if the chromosome complement consisted very largely of heterochromatic chromosomes. As was also observed in yeast by Levan (1946, 1947), the size and number of chromocentres is so closely paralleled by the mitotic chromosomes that there can be little doubt that most if not all of the chromosomes are highly heterochromatic. This would also account for their marked tendency to become closely aggregated and for the appearance of bridging strands of chromatin. The tendency of heterochromatin to coalesce is well-known and it has been demonstrated in widely different organisms, e.g. in *Drosophila*, *Cicer* (Thomas & Revell, 1946) and *Neurospora* (Singleton, 1953). The distribution of heterochromatin could not, of course, be determined in the minute chromsomes of *G. temulenta* but it is of interest to note that in *Neurospora crassa*, as in many higher organisms, it could be located in the centromere regions of each of the seven chromosomes (Singleton, 1953).

Meiosis and nuclear behaviour in the ascus have been studied in many ascomycetes and the general pattern of nuclear behaviour has been well established. Except in particularly favourable material (e.g. *Neurospora*), really detailed analysis of chromosome behaviour is not possible because of the minute size of the chromosomes. Nevertheless, there can now be little doubt that meiosis in fungi is essentially similar to that found in higher organisms. In *G. temulenta* it was not anticipated that nuclear behaviour in the ascus would show any marked deviations from that already described in other forms. The main purpose in investigating this stage was to provide a clear confirmation of the chromosome number found at mitosis ($n = 15$) and this was successfully achieved.

The nucleolus, it will be recalled, is a conspicuous feature of the vegetative nucleus. In higher organisms two major functions have been ascribed to this structure: (1) a role in the synthesis of cytoplasmic proteins, and (2) a role in chromosomal reproduction. It is important here to consider how far these functions can be ascribed to the nucleolus in *G. temulenta*.

The theory that the nucleolus plays an important part in protein synthesis is due to the work of Caspersson and his collaborators, and this has been reviewed in a monograph by Caspersson (1950). While this theory has been criticized on points of detail, the main conclusions appear to be valid (Brachet, 1955). Briefly, Caspersson's main conclusions are that the nucleolus and its associated heterochromatin are primarily responsible for the synthesis of RNA, which is essential for the synthesis of cytoplasmic proteins. High rates of protein synthesis can be shown to be regularly associated with high levels of RNA and usually with an enlarged nucleolus. It is therefore of particular interest that in *G. temulenta* the nucleolus undergoes changes in size which show a positive correlation with cytoplasmic basophilia (RNA) and that its maximum size is found in those cells which must be presumed to be actively engaged in protein synthesis, namely young vegetative hyphae and young asci. Similarly, in those cells with very little cytoplasmic RNA (e.g. microconidiophores) the nucleolus is either absent or so small as to be undetectable. Thus these observations are clearly in accord with Caspersson's view and suggest that fungal nucleoli have functions comparable with nucleoli in higher organisms.

Concerning the function of the nucleolus in nuclear reproduction, White (1951) has pointed out that most authors have tended to regard the nucleoli as temporary reservoirs of material which is transferred to the chromosomes before metaphase. It is difficult to reconcile this view with the behaviour of the nucleolus in *G. temulenta*; in the microconidiophores no nucleolus was ever observed yet there was active nuclear reproduction. Clearly in this instance the nucleolus can be dispensed with. It may well be, however, that the functions ordinarily residing in the nucleolus may be taken over by the heterochromatin, which is, in this organism, abundant. That this is possible is evident from the observations of Darlington (1947), who pointed out that when there is no organizer or nucleolus at all in an organism, there is always heterochromatin. He took the view that the nucleolus and the charged heterochromatin provide alternative sources of the nucleic acid used in the charging of the prophase chromosomes. The whole concept of the transfer of nucleolar material to chromosomes has, however, been challenged by Jain (1957) on cytological grounds and he has pointed out that all the recent experimental evidence shows that no nucleolar material, as such, is in fact transferred to the chromosomes. Also Borstel & Rekemeyer (1958) have shown that in *Drosophila* embryos, genetically deficient for nucleolar organizers, nuclear reproduction is unimpaired, clearly indicating the non-essential role of the nucleolus in chromosomal reproduction. The behaviour of the nucleolus in *G. temulenta* provides further support for this view and indicates again the parallelism between the role of the nucleolus in fungi and in higher organisms.

Role of nucleic acids in the differentiation of microconidia

The microconidia of *G. temulenta*, like those of other members of the Sclerotiniaceae, have never been induced to germinate (i.e. to produce germ tubes) and it has always been concluded that failure to induce germination could be attributed to the absence of suitable conditions (Arnaud & Barthelet, 1936). The fact that these spores are never required to *germinate* but only to fertilize appears to have been overlooked. When fertilization has been observed (e.g. in *Higginsia hiemalis*, Backus, 1934) there is no evidence of any tendency to germination on the part of the microconidium; it merely becomes attached to the trichogyne, the walls are dissolved at the point of contact and the nucleus migrates into the trichogyne through the pore thus produced. It cannot even be shown that the microconidium plays an active part in the dissolution of its own cell wall.

When viewed in this way the microconidia appear to lack any ability for normal vegetative growth processes and it may be asked whether their behaviour can be attributed to any differences in their cytological or cytochemical organization.

The most striking difference between microconidia and propagative spores (conidia and ascospores) is the low basophilia of the cytoplasm. It can be clearly demonstrated that the level of RNA in the cytoplasm is actually very low, and since RNA is essential for protein synthesis, the microconidia are, in comparison with propagative spores, very unfavourably placed for growth processes. This deficiency in RNA might thus well account for their behaviour. Further, the low cytoplasmic RNA appears even more significant when the development of the microconidia is taken into account. Since the cytoplasm of the microconidium is derived directly from that of the microconidiophore it is to be expected that low RNA values would be found in them also. This could obviously be accounted for by some mechanism regulating the rate of RNA synthesis in the microconidiophore. This, however, is unlikely in view of the following considerations. It has been noted that in the cells subtending the microconidiophores the amount of cytoplasmic RNA varies widely, from very high to zero. Further, as long as RNA could be detected in the subtending cells the microconidiophores invariably showed their normal low level. However, when zero levels were encountered in the subtending cells it was regularly associated with very low or zero levels in the attached microconidiophores. The simplest explanation to account for these observations is that the microconidiophore lacks ability to synthesize RNA and that the constant low level is maintained by withdrawal of RNA from the subtending cells; when this supply is exhausted nuclear reproduction and spore formation cease.

This explanation clearly raises several interesting problems relating to the origin and control of distribution of RNA. However, for the present argument it is sufficient to show that the low RNA in the microconidium is not fortuitous but is the result of an elaborate pattern of cytological and cytochemical differentiation. This situation contrasts sharply with that found in *Neurospora crassa*, in which the microconidia are capable of germination. Here, as might be inferred from consideration of *G. temulenta*, the microconidia are heavily charged with RNA.

These observations clearly have wider implications. Fundamental investigations on morphogenesis and differentiation have for the most part been conducted on complex organisms. In these it has been shown that nucleic acids play an important part in differentiation (Brachet, 1955). Consequently, it is of great interest that a similar role can be assigned to them in simple organisms such as fungi. Moreover, it suggests that fungi, because of their simpler organization and proven suitability for genetical and biochemical study, may provide valuable material for future studies on differentiation.

I am indebted to Prof. P. T. Thomas for much helpful advice and criticism, to Prof. D. G. Catcheside, F.R.S., for providing the culture of *Neurospora crassa* and to Dr E. C. Wright for providing samples of rye-grass seed naturally infected by *Gloeotinia temulenta*.

REFERENCES

Arnaud, G. & Barthelet, J. (1936). Les microconidies dans les genres *Sclerotinia*. *Bull. Soc. mycol. Fr.* **52**, 63–79.

Backus, M. P. (1934). Initiation of the ascocarp and associated phenomena in *Coccomyces hiemalis*. *Contr. Boyce Thompson Inst.* **6**, 339–379.

Barigozzi, C. (1949). A general survey of heterochromatin. *Portug. acta biol.* (Vol. R. B. Goldschmidt), pp. 593–620.

Barratt, R. W. & Garnjobst, L. (1949). Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. *Genetics*, **34**, 351–369.

Beauverie, J. & Guillermond, A. (1903). Étude sur la structure du *Botrytis cinerea*. *Zbl. Bakt.* **10**, 275–281, 311–320 (cit. Heuberger, 1934).

Bjorling, K. (1942). Undersökningar Rörande Klöverrötan II. Studier av Utvecklingshistoria och variation hos *Sclerotinia trifoliorum*. *Medd. Växtskyddsanst. Stockh.* **37**.

Borstel, R. C. & Rekemeyer, M. L. (1958). Division of a nucleus lacking a nucleolus. *Nature, Lond.*, **181**, 1597–1598.

Brachet, J. (1955). The biological role of the pentose nucleic acids. In *The Nucleic Acids*, II (ed. Chargaff and Davidson), pp. 475–579. New York.

Caspersson, T. O. (1950). *Cell growth and cell function.* New York.

Catcheside, D. G. (1951). *The genetics of micro-organisms.* London.

Darlington, C. D. (1947). Nucleic acid and the chromosomes. *Symp. Soc. exp. Biol.* **1**, 252–269.

Darlington, C. D. & La Cour, L. F. (1947). *The handling of chromosomes*, 2nd ed. London.

Drayton, F. L. (1934). The sexual mechanism of *Sclerotinia gladioli*. *Mycologia*, **26**, 46–72.

Drayton, F. L. (1937). The perfect stage of *Botrytis convoluta*. *Mycologia*, **29**, 305–318.

Faberge, A. C. (1945). Snail stomach cytase, a new reagent for plant cytology. *Stain Tech.* **20**, 1–4.

Griffiths, E. (1958). Sexual reproduction and variation in *Gloeotinia temulenta* (blind seed disease of ryegrass). *Trans. Brit. mycol. Soc.* **41**, 461–482.

Groves, J. W. & Drayton, F. L. (1939). The perfect stage of *Botrytis cinerea*. *Mycologia*, **31**, 485–489.

Harper, R. A. (1905). Sexual reproduction and the organisation of the nucleus in certain mildews. *Publ. Carneg. Instn*, **37**, 1–104 (cit. Singleton, 1953).

Heuberger, J. W. (1934). Fruit-rotting Sclerotinias. IV. A cytological study of *Sclerotinia fructicola* (Wint.) Rehm. *Md. agric. Exp. Sta.* **371**.

Jain, H. K. (1957). Effect of high temperature on meiosis in *Lolium*: Nucleolar inactivation. *Heredity*, **11**, 23–36.

Johansen, D. A. (1940). *Plant microtechnique*. New York.

Korson, R. (1951). A differential stain for nucleic acids. *Stain Tech.* **26**, 265–270.

Levan, A. (1946). Mitotic disturbances induced in yeast by chemicals and their significance for the interpretation of the normal chromosome condition in yeast. *Nature, Lond.*, **158**, 626.

Levan, A. (1947). Studies on the camphor reaction in yeast. *Hereditas*, **33**, 457–514.

Lewis, K. R. (1956). A cytological survey of some lower organisms with particular reference to the use of modern techniques. Ph.D. Thesis, University of Wales.

Pinto-Lopes, J. (1949). Contributions to the study of the nuclear structure of fungi. *Portug. acta biol.* **2**, 191–210.

Pontecorvo, G. (1949). Auxanographic techniques in biochemical genetics. *J. gen. Microbiol.* **3**, 122–126.

Serra, J. A. (1947). Données pour une cyto-physiologie du nucleotide. *Portug. acta biol.* **1**, 51 (cit. Pinto-Lopes, 1949).

Singleton, J. R. (1953). Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. *Amer. J. Bot.* **40**, 122–144.

Sloan, B. J. & Wilson, G. B. (1958). The functions of the microspores of *Gelasinospora calospora*. *Mycologia*, **50**, 111–116.

Swift, H. (1955). Cytochemical techniques for nucleic acids. In *The Nucleic Acids*, II (ed. Chargaff and Davidson), pp. 51–92. New York.

Thomas, P. T. (1940). The aceto-carmine method for fruit material. *Stain Tech.* **15**, 167–172.

Thomas, P. T. & Revell, S. H. (1946). Secondary association and heterochromatic attraction. I. *Cicer*. *Ann. Bot., Lond.*, **10**, 159–164.

White, M. J. D. (1951). Nucleus, chromosomes and genes. In *Cytology and cell physiology*, 2nd ed. Oxford.

Wilson, I. M. (1952). The ascogenous hyphae of *Pyronema confluens*. *Ann. Bot., Lond.*, **16**, 322–339.

EXPLANATION OF PLATES 7 AND 8

Plate 7

Fig. 1. Resting nucleus in a microconidiophore showing prochromosomes but no nucleolus. × 3200.

Figs. 2, 3. Resting nuclei in a hypha and conidiophore, respectively, showing in each a prominent nucleolus in addition to the prochromosomes. × 3200.

Fig. 4. Mitotic metaphase in a microconidiophore showing 15 chromosomes. × 4800.

Fig. 5. Explanatory drawing of fig. 4.

Figs. 6–8. Stages in mitosis in the microconidiophore. Fig. 6, metaphase; fig. 7, anaphase; fig. 8, telophase. All × 2800.

Figs. 9–12. Stages in spore development. Fig. 9, daughter nucleus passing into the microconidial bud; fig. 10, condensed daughter nuclei in young spore and microconidiophore; fig. 11, the last nucleus passing into a spore; fig. 12, a later stage. Note in figs. 11 and 12 the absence of basophilia in the cytoplasm. All × 2800.

Fig. 13. Group of microconidiophores showing variation in cytoplasmic basophilia. × 2800.

Figs. 14, 15. Teased fragments of microconidial sporodochia stained with methyl green and toluidine blue. Fig. 14, note high basophilia (= high RNA) in subtending cells and low basophilia in microconidiophores. The cytoplasm of the microconidia is very lightly stained. Fig. 15, subtending cells unstained. Note that only one of the six microconidiophores shows any cytoplasmic basophilia. × 1200.

Figs. 16, 17. Microconidiophores and microconidia of *Neurospora crassa* stained with methyl green and toluidine blue. Note the high basophilia in both microconidiophores and microconidia. × 880.

PLATE 8

Fig. 1. Section through an ascogonial stroma on the surface of an infected seed showing the coiled hypha (ascogonium) at the centre of the stroma. × 360.

Fig. 2. Longitudinal section through a young apothecium showing darkly staining hyphae in the central part of the stroma. × 120.

Figs. 3–5. Stages in ascus development. × 2800. For explanation see Text-fig. 3.

Fig. 6. Metaphase I of meiosis, showing 15 bivalents. × 4800.

Fig. 7. Explanatory drawing of Fig. 6.

Fig. 8. Metaphase II of meiosis showing 15 chromosomes. × 4800.

Fig. 9. Explanatory drawing of Fig. 8.

Figs. 10–13. Stages in post-meiotic nuclear development. All × 2800. Fig. 10, four-nucleate stage; diffuse interphase nuclei. Fig. 11, metaphase III; chromosomes clumped together. Fig. 12, eight-nucleate stage; telophase of division III. Fig. 13, eight-nucleate stage; nuclei becoming diffuse and showing spindle-shaped areas with fibrillar structure.

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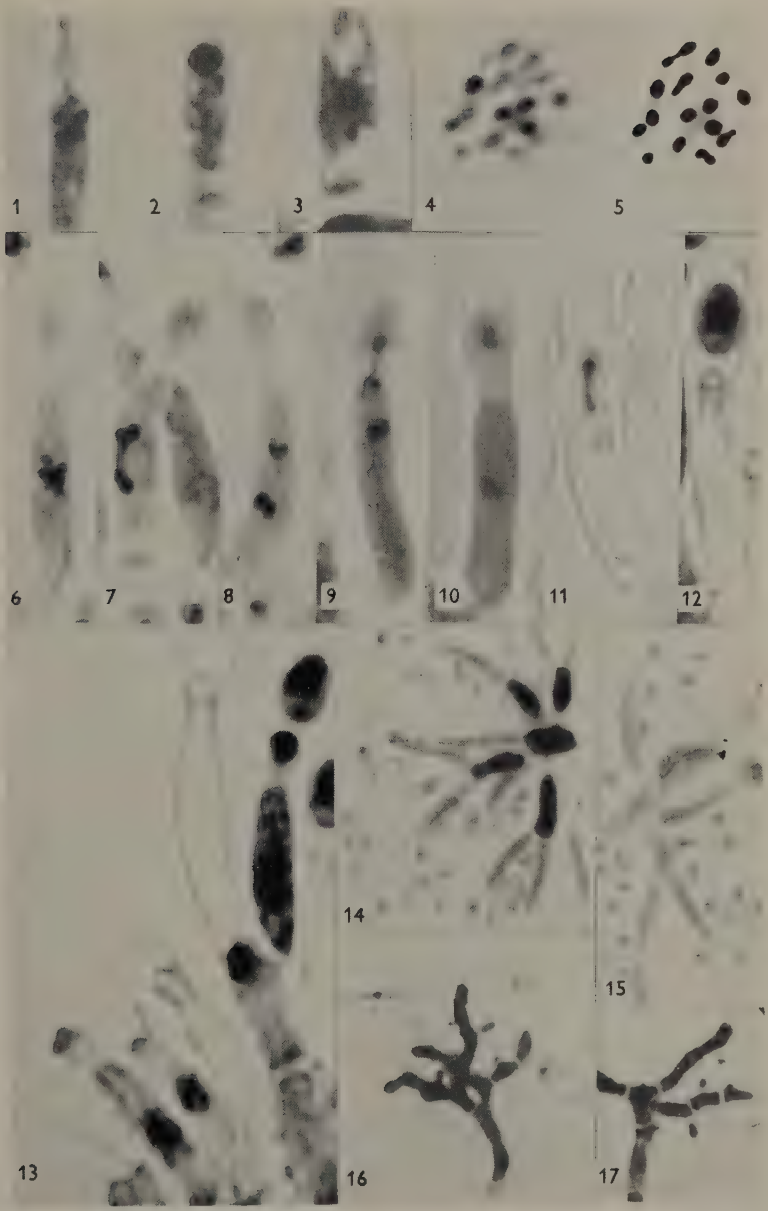
A correction. I wish to apologize for incorrectly citing the name *Gloeotinia temulenta* in *Trans. Brit. mycol. Soc.* **41**, 461–482 (1958). The citation should read *Gloeotinia temulenta* (Prill. & Delacr.) Wilson, Noble & Gray, not *Gloeotinia temulenta* (Prill. & Delacr.) Wilson & Gray. E.G.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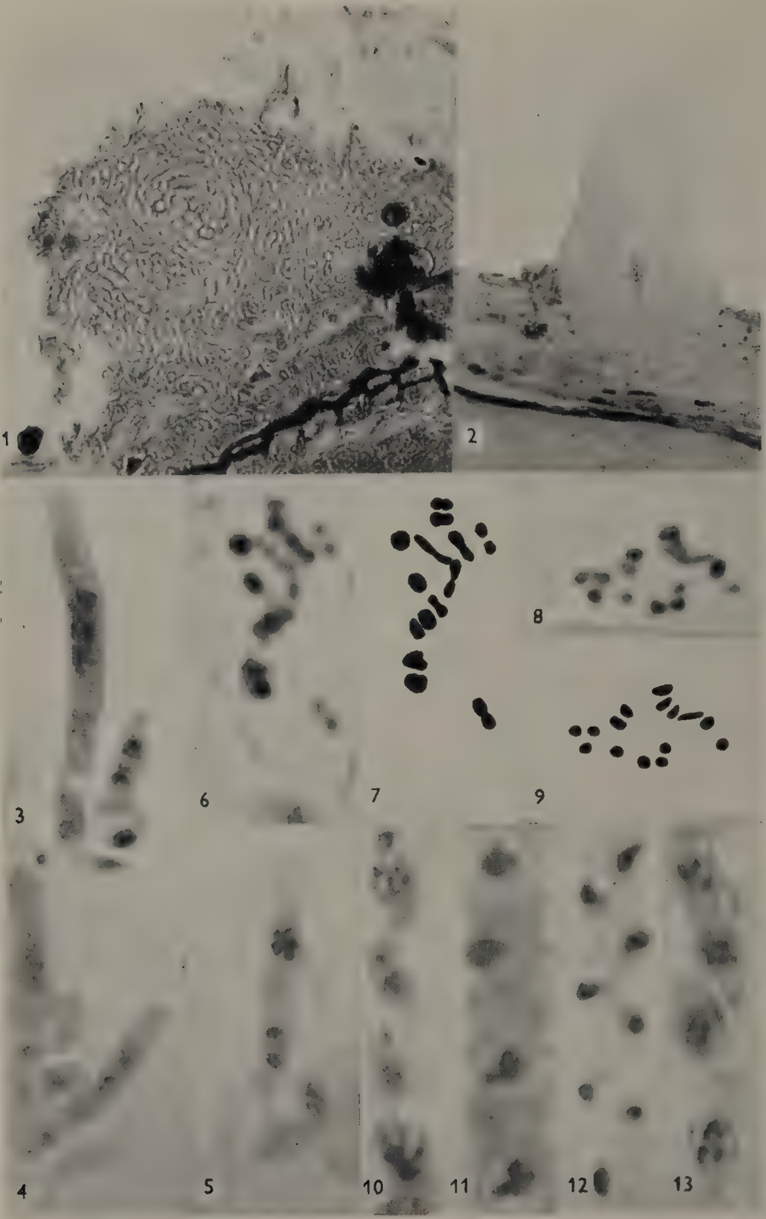
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Trans. Brit. mycol. Soc. 42 (2), 149–173 (1959)

THE GENUS *EPICOCCUM* LINK

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(With Plate 9 and 4 Text-figures)

A study has been made of 70 isolates of *Epicoccum*, 47 of which sporulated, and also of 19 type specimens and 96 other herbarium specimens.

The 70 strains cultured under normal laboratory conditions are red, yellow or olivaceous in different shades. Colour, however, cannot be used as a dependable taxonomic character because it is too much influenced by various factors such as medium, light and pH.

The pluricellular structure of the ripe spore has been proved by aid of microtome sections; the spores germinate with many germ tubes. The number of the cells in a spore varies up to fifteen.

The thickness of the epispore, which causes the roughness of the spore, and that of the spore wall, the shape and size of the spore, even in one and the same strain, and the size of the sporodochia are far too variable to be of specific value.

A wide divergence appears in the spore measurements of 47 strains. A thorough examination of herbarium material led to the conclusion that no real difference exists between those species which proved to belong to *Epicoccum*. They could all be reduced to synonymy with the type, *E. nigrum* Link, together with the 70 strains in culture.

Eight species are considered doubtful or must be eliminated as not belonging to the genus *Epicoccum*.

The genus *Cerebella* should be discarded; the only species is included in *Epicoccum* as *E. andropogonis* (Ces.) nov.comb.

Over the years, the Centraalbureau voor Schimmelcultures has received for identification a number of fungi belonging to the genus *Epicoccum*. The great variability observed between different strains and even in a single strain made the study of this genus necessary.

THE GENUS *EPICOCCUM* LINK

Link (1815) gave the following diagnosis: '*Epicoccum*. Stroma globosum compactum sporidiis subglobosis inspersis. Sporidia hinc inde adspersa incumbunt, nec undique tegunt ut in Dermosporio, nec instar densi strati incumbunt, ut in Tubercularia.'

The type species, *E. nigrum* Link, was given the following diagnosis: '*E. nigrum*, macula effusa nigra, stromatibus globosis minutis aggregatis nigris. In caulibus plantarum aridis Rostockii, nec non Vratislaviae. Macula nigra caulem obtegit, in qua granula minuta nigra pulveris pyrii granis minora aut sparsa aut conferta eminent.'

This diagnosis is very unsatisfactory, nothing being said whether the type has continuous or multicellular spores. This has led to much confusion.

About 60 species of *Epicoccum* have been described of which Lindau (1910) mentions 26. Most of the diagnoses are incomplete, often without spore measurements. The diagnoses of species described in later decades are also very unsatisfactory. All these descriptions are based on characters of the fungus as it occurs in its original habitat. Many of the mentioned differences such as occurrence on spots, the colour, the shape of the spore and the occurrence of a pedicel, mentioned in old diagnoses, have proved not to be of specific value. As data on spore sizes are often lacking, it is clear that most of these species are ill defined. Saccardo (1886) gives *E. vulgare*, *E. versicolor*, *E. purpurascens*, *E. neglectum*, *E. menispermi*, *E. granulatum* as synonymous with *E. nigrum*.

According to Lindau (1910), *Epicoccum* belongs to the section Amerosporae of the Tuberculariaceae dematieae, although his diagnosis mentioned conidia often with a reticulate surface, unicellular or divided by septa. Lindau stated that the conidia of the type species *E. nigrum* are reticulate.

The reticulate surface recorded for some species suggested pluricellularity of the spores, but the variability in occurrence of this reticulate structure caused confusion. Therefore von Höhnel (1910) proposed to divide the genus into species with 1-celled and species with multi-celled spores and to range the latter under *Thyrococcum*. Lindau, however, objected, because it was not known whether the 1-celled spore would ripen and become many-celled. This being the case he suggested first of all the investigation of the germination of the conidia.

For some species it was indicated that the dividing walls in a spore meet in one point, with the consequence that the cells in the spore are pyramidal with their apices in the centre of it.

Massee (1906) wrote that the genus *Epicoccum* stands in need of revision, as some species are described with continuous and others with pluricellular spores.

Buchanan (1911) published an article concerning a hyphomycete which he at first referred to the genus *Epicoccum*. After he had proved the septation of its spores, on this basis and following Lindau, he named the fungus *Thyrococcum humicola*. Saccardo (1931), however, changed the name into *E. humicola* (Buch.) Sacc.

Nicolas & Dop (1926) mentioned unicellular as well as some pluricellular spores of *E. nigrum*.

Saccardo (1931) did not clarify the situation by creating the subgenera *Eu-Epicoccum* for non-clathrate and *Dictyococcum* for clathrate forms, meanwhile keeping the genus within the Amerosporae.

Goidanich (1937) described the pluricellularity of the conidia of *E. granulatum*.

Wakefield & Bisby (1941) finally grouped *Epicoccum* with the Dictyosporae.

MATERIALS AND METHODS

I studied the structure of the spore by aid of microtome sections, by an extensive examination of herbarium material and also in preparations made from pure cultures on different media.

Further, an attempt was made to seek specific characters by which it might be possible to distinguish the _Epicoccum_ species. Because spore sizes in this genus range from 6 to 65 μ, extensive spore measurements had to be made. Furthermore, the question was studied whether the characters observed in pure culture on different media could be used to distinguish the species.

Since 1949, 70 isolates of _Epicoccum_ from nineteen countries have been obtained and cultured (see Table 1). Only 47 of them—marked with an asterisk—produced enough spores to be used for measurements.

In nature, the fungus occurs as little dark pustules generally on dead parts of various plants. It is also rather common on mouldy paper or textile and it has been more than once isolated from the air, insects, human skin and sputum. Sometimes it has a more or less parasitic character. Often the pustules develop on spots which may be coloured. The pustules are sporodochia on which the spores are formed.

Table 1. _List of strains of_ Epicoccum _at the C.B.S._

(The strains marked with an asterisk were used for spore measurements.)

Epicoccum heterochroum ad int., sent by Wollenweber, from potato, Germany, 1922
E. humicola (Buch.) Sacc., Tucker, no. 43, U.S.A., 1931
*_E. granulatum_ Penz., Heald, U.S.A., 1932
E. granulatum Penz., Robak, from wood pulp, Norway, 1932
*_E. purpurascens_ Ehrenb., Jollyman, from tobacco no. 34, Britain, 1932
E. neglectum Desm., Jaarsveld, from wheat grains, the Netherlands, 1933
E. oryzae Ito & Iwadare, authentic material, Ito, no. 1 from rice, Japan, 1934
*_E. oryzae_ Ito & Iwadare, authentic material, Ito, no. 2 from rice, Japan, 1934
E. mezzettii G. Goid., _type_, Goidanich, from poplar wood pulp, Italy, 1938
Mulder 1, Mulder
Mulder 17, Mulder } from apple leaves, the Netherlands, 1948
*_Mulder_ 21, Mulder
*_Mulder_ 41, Mulder, from pear buds, the Netherlands, 1948
*_van Vloten_, van Vloten, from twig of _Alnus glutinosus_, the Netherlands, 1949
*_Orie_, Orie, from sputum, the Netherlands, 1949
*_Mansens_, Mansens, from sputum, the Netherlands, 1949
*_Leiden_, van den Honert, the Netherlands, 1949
*_van Holder_, van Holder, common on different substrata, Belgium, 1949
Lobry de Bruyn no. 1
*_Lobry de Bruyn no._ 2
*_Lobry de Bruyn no._ 3 } Schol-Schwarz, from canvas, the Netherlands, 1949
Lobry de Bruyn no. 4
*_Lupinus_, Plantenziektenkundige Dienst, from _Lupinus_, the Netherlands, 1949
cauliflower, Plantenziektenkundige Dienst, no. e418 from cauliflower, the Netherlands, 1950
*_Q._ 47, Quintanilha, from cotton seed, Africa, 1949
*_Q._ 66, Quintanilha, from cotton, Africa, 1950
*_Verona_, Verona, Italy, 1950
*_Portugal A_
*_Portugal C_ } Loureiro Ferreira, Portugal, 1950
*_Portugal D_
Ordman A
*_Ordman C_ } Ordman, from the air, S. Africa, 1951
Olivier, Olivier, S. Africa, 1951
*_Baruah_, Baruah, from _Aquilaria agallocha_, India, 1951
Koopmans, Koopmans, from potato leaf, the Netherlands, 1951
_C.B.S._, C.B.S., from the air, the Netherlands, 1951
*_Cajkovac_, Cajkovac, from human skin, Yugoslavia, 1952
*_E. vulgare_ Corda, Nicot, from _Opuntia_, France, 1952
*_E._ sp., Nicot, from twig of _Aleurites_, France, 1952
*_Bergen_ 44, Eilertsen, Norway, 1952

Table 1 (*cont.*)

Geigy 8
Geigy 10 } Hentrich, from apple buds, Switzerland, 1952
Finland, Jarmunen, Finland, 1952
**Red currant*, Leefmans, from feeding galleries of *Sesia tipuliformis* in twigs of red currant, the Netherlands, 1952
**de Vries Mulder*, de Vries, from man, the Netherlands, 1953
**Irtel*, Irtel, from a parasite on *Lecanium*, Turkey, 1953
**van Beverwijk*, van Beverwijk, from a decaying leaf in a pool, the Netherlands, 1953
**Küster* 5-I-3, Küster, from peat soil pH 3·1 at 10 cm. depth, Hanover, Germany, 1953
Küster 5-III-2, Küster, from peat soil pH 2·94 at 50 cm. depth, Hanover, Germany, 1953
E. purpurascens Ehrenb. I 4037
E. purpurascens Ehrenb. I 4160 } Nagao Institute, Japan, 1954
**Witkamp*, Witkamp, from oak leaf, the Netherlands, 1954
**de Areâ Leao*, de Vries, from man, Brazil, 1954
**Frico*, Frico, from butter, the Netherlands, 1954
**J. Smit*, J. Smit, the Netherlands, 1954
**New Guinea*, Hueck, from a mouldy shirt, New Guinea, 1954
**Koelhuis*, Houthuis, from a mouldy wall, Rotterdam, 1954
**Espagne* 6, Nicot, Spain, 1955
**Loire* 2, Nicot, France, 1955
**Becker* 1
Becker 2 } Becker, from wheat grains, the Netherlands, 1955
**F* 419
R 109 } Meyer, S. Africa, 1955
**beach chair*, Hueck, from a beach chair, the Netherlands, 1955
**Cooke* 724, Cooke, from the air, U.S.A., 1956
**Cooke* 1842, Cooke, from the air, U.S.A., 1956
**Cooke* 2040, Cooke, from the air, U.S.A., 1956
**Cooke* 2119, Cooke, from sewage treatment plant effluent, U.S.A., 1956
**Cooke* 2359, Cooke, from experimental trickling filter stone, U.S.A., 1956
**Cooke* 2388, Cooke, from sewage treatment plant sludge, U.S.A., 1956

Cultural characters

The collection of 70 strains was kept in duplicate on cherry agar and oatmeal agar on open racks at room temperature.

The fungus grows very well on different media, such as cherry, oatmeal, Czapek Dox and Sabouraud glucose agars and sterilized stalks of *Lupinus polyphyllus*. The abundantly developed mycelium is characterized by various colours, the range of which varies from white to pink, red, purple, yellow and more rarely from olivaceous green to brown. In one tube different colours may occur. The pigment also diffuses into the agar but the colour of the mycelium and that of the medium are not always the same. Often the media are more pigmented than the mycelium. The cultures on stalks of *Lupinus* are mostly very poorly coloured, whereas on media rich in carbohydrates such as oatmeal and Sabouraud glucose agars, the colours are vivid. Among them purple, red and bright yellow are dominant. Cultured under normal laboratory conditions some strains are known as red, some as yellow and others as olivaceous ones.

In the course of the years it appeared that the red strains do not change much in colour, but that the pigment development of the bright yellow strains tends to decline. The colour of the dark olivaceous strains is rather stable, whereas the mycelium of the light olivaceous strains tends to become hyaline. Sometimes brightly coloured drops of liquid are observed on the mycelium. Some strains, when plated, show differently coloured sectors. Transfers from those different sectors do not yield permanently different coloured strains.

Sporulation is erratic. Some strains fructify very easily, when freshly isolated, but after prolonged culture spore development tends to decline; other strains are very reluctant in developing spores and special culture conditions must be tried to induce fructification. The most favourable medium for sporulation is sterilized stalks of *Lupinus polyphyllus*.

Sometimes transfers from very dry cultures of 8 or more months old on *Lupinus* give well-sporulating cultures. Transfers kept at a few degrees above zero for some weeks may begin to fructify soon after being returned to about 20° C.

Of two authenticated strains of *E. oryzae* Ito & Iwadare, sent by Ito from Japan and cultured at the Centraalbureau since 1934, one never sporulates even on very poor media or on stalks of *Lupinus*, whereas the other never fails to fructify.

Most of the newly isolated strains show a rich development of aerial mycelium which gives the culture a fluffy appearance. This character changes at the time of fructification and the mycelium begins to develop little tufts in which sporodochia shortly develop. At that time in new transfers the development of aerial mycelium declines and more sporodochia appear. Well sporulating transfers on sterilized stalks of *Lupinus* are characterized by an almost total lack of aerial mycelium and by many sporodochia (Pl. 9, fig. 10).

THE SPORE

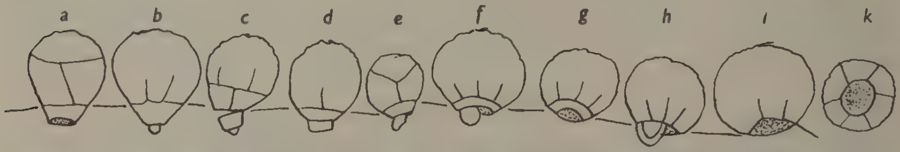
The spores in the genus *Epicoccum* are very characteristic. They are globose, subglobose, pyriform, sometimes angular, more or less granular verrucose, especially at the distal end, and areolate. Most of them are golden brown but they vary to brown, sometimes olivaceous, even to very dark brown or black. The spores are aleuriospores and they are normally produced on sporodochia. Sporodochia are built up of conidiophores on a pseudoparenchymatous stroma (Pl. 9, fig. 1). In culture conidiophores originate as swollen side branches of the mycelium, at first occurring irregularly (Pl. 9, fig. 2), gradually growing out into dense bundles (Pl. 9, figs. 3, 4). These bundles consist of hyaline, unbranched, smooth conidiophores originating from a stroma (Pl. 9, fig. 4). This stroma is more or less globose in soft media, such as agar, and hemispherical on solid media, such as sterilized stalks of *Lupinus*, and on natural substrata, such as various parts of decaying plants. The top of a young conidiophore is fuscous and develops into a single dark aleuriospore. Pl. 9, fig. 1, shows a section of a sporodochium with conidiophores of different ages; a young one bears a spore, others are shrivelled after having shed their spores. Very young sporodochia can be recognized from the fuscous tops of the sporiferous hyphae.

I have seen unpublished notes prepared in 1945 by S. J. Hughes; according to his account a cross-wall, formed in a young sporiferous hypha, cuts off the spore initial from the stalk cell. This wall remains entirely unthickened and hyaline. The next wall laid down is a transverse wall in the lower part of the initial, cutting off a small *basal cell* next to the stalk cell. Hughes emphasized that the development of the basal cell is the quintessence in the spore development of *E. purpurascens*:

> This basal cell varies in shape and size but it is a constant feature in all aleuriospores which have been observed in the process of developing. The basal cell remains undivided and is less coloured and less verrucose than the upper globose part of the spore. The upper swollen part of the spore in the meantime develops cross-walls which, however, are not at all easy to follow. The first is generally vertical though sometimes oblique; further walls are formed to cut up the spore into a compact ball of thick-walled cells.
>
> In by far the greatest number of cases the freeing of the spores occurs by a dehiscence at the junction of stalk and basal cell.
>
> The newly exposed and unthickened end of the basal cell often protrudes a little from the outer wall layer....Less frequently the swollen end of the aleuriospore separates from the basal cell which is left behind attached to the stalk cell. Sometimes the unthickened apex of the stalk cell continues its growth radially to push away the basal cell of the spore. Rupture of this prolongation can occur, with the result that the basal cell of the detached spore bears a hyaline, collapsed and, consequently, narrower collar of the hyaline cell wall attached to its base.

The process as described by Hughes has been observed in every strain of *Epicoccum* which I have studied.

Ellis (1956), too, described the development of the basal cell, but he did not call it so, nor did he emphasize its development.



Text-fig. 1. The basal cells lie beneath the connecting line. *a*, Young spore with a conical basal cell and a clear scar; *b*, young spore with a high narrow basal cell and protruding septum; *c*, young spore with a dehiscing basal cell with protruding septum; *d*, the basal cell becomes ring shaped, collar present; *e*, the basal cell has an irregular collar; *f*, ripe spore with a ring-shaped basal cell and a dehiscing collar; *g*, ripe spore with a ring-shaped basal cell and a clear scar; *h*, ripe spore with a dehiscing ring-shaped basal cell; *i*, ripe spore without basal cell and with a big scar; *k*, ripe spore without basal cell, showing the big scar.

The basal cell of an unripe spore is conical and not much narrower than the upper globose part of the spore (Text-fig. 1 *a*). Text-fig. 1 *b*, *c* shows spores with the septum somewhat protruding at the unthickened lower wall of the basal cell. As the spore ripens, the upper globose part expands, but the basal cell does not grow to the same extent. It is drawn out until, in the ripe spore, it can be seen at the base of the globose spore only as a flat small ring, surrounding the scar where the spore was attached to the stalk cell (Text-fig. 1 *g*). The basal cell may separate from the upper globose part of the spore (Text-fig. 1 *h*; Pl. 9, fig. 5) and then the scar at the base is much larger (Text-fig. 1 *i*, *k*). Moreover, a spore which has kept its basal cell may show a hyaline collar of irregular shape formed by rupture of the upper part of the wall of the stalk cell (Text-fig. 1 *d*, *e*). Thus many different forms of spores are to be found in every preparation (Text-fig. 1, *a*–*k*).

In the older literature the presence of a pedicel on the spore was used as a specific character. According to the descriptions given above, it is most probable that the name pedicel was used for the conical basal cell in young spores (Text-fig. 1 *a*), as well as for the hyaline collar of riper conidia

(Text-fig. 1 *d*, *e*). It is clear now that a pedicel is not a character of specific value.

In the globose upper part of the young spore the first developed septa are usually vertical (Text-fig. 1 *b*), but sometimes oblique (Text-fig. 1 *a*). More septa develop in various directions, until the ripe spore is a compact ball of irregular thick-walled cells, the number of which may vary up to 15. This process is not always easy to follow. Ripe spores with a rather thin outer wall show on the surface a reticulate, so-called areolate structure, but the outer wall may be too thick for this to be seen. This has caused the above-mentioned controversy in the literature concerning the continuous or multicellular nature of the spores in *Epicoccum*.

Buchanan (1911) was the first to describe the spores as multiseptate, and he observed that the spores germinate with many germ tubes. Goidanich (1937) affirmed the pluricellularity of spores of *E. mezzettii*. He illustrated the first stages of spore development with the basal cell but he did not recognize that the basal cell is the quintessence, as Hughes emphasized.

I studied the inner structure of the spore by means of microtome sections. Ripe sporodochia and spores were mounted in paraffin and sectioned at 10 μ. Every ripe spore consists of up to fifteen rather thick-walled cells, which in the sections appear clearly as cells with brown walls and contents which stain blue in lactic acid tinged with cotton blue. Sometimes a middle lamella is seen in the septum between the cells of a spore. The thickness of the septa diminishes towards the centre of the spore. It is very likely that septation originates from the outer spore wall (Pl. 9, fig. 6).

Lindau's (1910) assumption that the septa co-ordinate towards the centre of the spore, giving the cells a pyramidal shape, could not be confirmed. Nor was a radial arrangement of the septa as described by Buchanan (1911) observed. The cells within the spore are irregularly shaped.

A sporiferous hypha is distinguished by a fuscous, rounded top with a thickened wall. During spore development the wall, which consists of different layers, increases in thickness, particularly at the distal end. When the upper part of the spore swells, the outer dark layer of the wall, or epispore, cracks and splits up in small pieces or warts of different shape and thickness. The spore is most verrucose at its apex, the thickness of the epispore diminishing towards the base. The wall of the basal cell is only slightly coloured and cracked and the ring-shaped basal cell of ripe spores is often lighter in colour and much smoother than the globose upper part of the spore.

The areolate surface and the inner septation can easily be observed in spores of freshly fructifying strains and also in spores of old herbarium material when heated in 7 % KOH, lactic acid being added after some minutes.

Spores with a conspicuous reticulation were observed, as well as such without a conspicuously areolate surface, in every specimen of herbarium material examined, even in the specimens of 'species' described with continuous spores as well as in the 47 cultured sporulating strains.

Under favourable circumstances every spore germinates with several germ tubes. Even the basal cell can germinate.

The shape of the spores is very variable; they may be globose or more or less angular, some ripe spores are longer than broad, others again more broad than long. In every preparation made of living strains or of herbarium specimens, spores of these different shapes occur. So the shape is not a character of specific value.

Pl. 9, figs. 7–9, show a sporodochium and spores of the type *E. nigrum.*

In view of the variability of the spore, it is of greatest importance that, for comparing different strains, only spores with a flat ring-shaped basal cell (Text-fig. 1 *g*), which are supposed to be ripe, are used.

THE SPORODOCHIA

The size of the sporodochia is very variable in the different cultured strains. It was observed that it changed in the course of the years and on different media. Of two easily fructifying strains, *Cooke* 2040 normally has large sporodochia up to 2000 μ (Pl. 9, fig. 10*c*), while *Cooke* 1842 has very small sporodochia (Pl. 9, fig. 10*a*), *c.* 100 μ. Of each strain four single spore cultures were made. In the beginning all those of *Cooke* 2040 produced large sporodochia, while all those of *Cooke* 1842 produced very small ones. However, Pl. 9, fig. 10*b* shows one of the single-spore strains of *Cooke* 2040 on a sterilized stalk of *Lupinus.* In this culture large sporodochia developed on the upper half of the stem (right), whereas small sporodochia grew on the submerged lower half (left). The colour of the sporodochia varies from brown to black.

THE PIGMENT

The production of the pigment of *Epicoccum* has been studied by different authors, especially by Naumann (1912), who investigated a single strain of *E. purpurascens* and stated that the pigment development is dependent on the presence of Mg^{++} and NO_3^-. Maltose and starch are the most favourable carbohydrates; the optimum temperature is 21–26° C., and no pigment occurs at 30° C. According to Naumann, the development of pigment is more abundant when the culture is kept in the dark. The red pigment of the mycelium is soluble in methyl alcohol, ethyl alcohol and alcohol-ether. In alcohol it soon changes irreversibly to red-brown when in contact with the air; in alcohol-ether it keeps for some weeks. Naumann considered the pigment as an indicator which is yellow in an acid and red in an alkaline medium.

C. & M. Moreau (1951) also investigated the pigment of a single strain called *E. purpurascens*, which they cultured in maize decoction of different pH values for 10 days. When the pH was 6·8 or 5·6 after filtration they obtained a yellow liquid. No growth occurred at pH 8·1. With a trace of sulphuric acid a red precipitate was formed in the yellow liquid which disappeared after adding more acid, while the liquid turned drab. With a trace of ammonia the yellow liquid turned citron yellow or yellowish white. According to C. & M. Moreau the pigment of their strain of *E. purpurascens* was red in acid and yellow in alkaline media, which is just the reverse of Naumann's statement. It was soluble in water and acetone, very soluble in alcohol and insoluble in benzol and toluol.

In the present investigation the question arose whether the pigment can be considered as a character of specific value, and how far the appearance of a special colour is influenced by cultural conditions.

Influence of light

Series of newly made transfers on oatmeal agar of three strains, namely, *Portugal C*, a red strain, *Cooke* 724 which is yellow under normal laboratory conditions, and *Mulder* 1, a dark olivaceous strain, were placed in very bright daylight in front of a south window in May at a temperature varying from 20° to 30° C. After 5 days the agar in all these cultures was red, more intensely so in those of *Portugal C* than in those of *Cooke* 724; the agar was red-brown in cultures of *Mulder* 1. The mycelium in the cultures of *Portugal C* was red, in those of *Cooke* 724 pink and in those of *Mulder* 1 dark olivaceous.

Thus, cultures of *Cooke* 724 and of *Mulder* 1 producing a red pigment became available. Transfers of these as well as of the originally yellow *Cooke* 724, dark olivaceous *Mulder* 1 and red *Portugal C* were placed in the dark at 25° C. After 3 days all the transfers had their original habit, i.e. those of *Portugal C* had red mycelium and red agar, those of *Cooke* 724 had yellow mycelium and yellow agar and those of *Mulder* 1 had dark olivaceous mycelium and olivaceous brown agar. No differences could be observed between the ordinary cultures and those transferred from the cultures grown in very bright daylight. This means that the exposure of the transfers to very bright daylight induces the production of red pigment in the agar.

All the 70 strains were tried in the same way and it appeared that the red pigment is potentially present in every strain. Artificial light, when strong enough, has the same effect. Compared with cultures exposed to very bright daylight, those grown in the dark show more conspicuous pigmentation.

Another series of experiments, to study the pigment which diffuses into the liquid medium, was performed with the red strain *Portugal C*, the yellow *Cooke* 724 and the light olivaceous *Mulder* 21. They were grown in Erlenmeyer flasks in oatmeal decoction with and without 0·1 % $Mg(NO_3)_2$, to check the conclusion of Naumann (1912) who stated that the development of pigment in his strain was influenced by the presence of Mg^{++} and NO_3^-. One series in duplicate was kept in very bright daylight and one in the dark at 25° C. After 11 days the liquid was filtered off and treated with ammonia and sulphuric acid in traces as well as in excess. An obvious influence of $Mg(NO_3)_2$ on the colour of the mycelium could not be detected. The colour of the filtrate of *Mulder* 21 was somewhat darker with $Mg(NO_3)_2$ than without. However, the filtrate of *Portugal C* grown in the dark with $Mg(NO_3)_2$ showed just the opposite result. The influence of the light appeared from a tinge more red in the filtrate compared with that kept in the dark. But in no case was the filtrate of oatmeal decoction cultures grown in very bright light really red, in contrast with the colour of oatmeal agar, which is always red in cultures grown in very bright daylight as has been said before.

The filtrate of the 3 strains in general became lighter with excess of acid, but it reacted differently with ammonia. In these experiments often excess of acid and excess of ammonia gave the same result.

A red solution could be obtained by extracting the red mycelium of *Portugal C* with alcohol. This solution turned golden yellow with a trace as well as with excess of acid. With a trace of ammonia the colour turned less red but with excess of ammonia it became golden yellow. Here also acid as well as ammonia in excess had the same result. So these experiments do not confirm the opinion of Naumann (1912) that the pigment of *Epicoccum* simply reacts as an indicator which is yellow in an acid and red in an alkaline medium.

Influence of pH

Series of transfers of the red strain *Portugal C*, the yellow *Cooke* 724 and the dark olivaceous *Mulder* 1 were made on oatmeal agar of different pH, i.e. alkaline 8·85, normal 6·85, and acid 3·1. One duplicated series was placed in very bright daylight and another in the dark at 25° C. In every case the pigmentation of the mycelium, as well as that of the agar, was most conspicuous on alkaline oatmeal, somewhat less on normal and still less on acid, thus declining in intensity with decreasing pH. The same results were obtained with other strains. The influence of the light was the same as described on p. 157.

The results of the experiments indicate that the observed colour in the different strains cannot be used as a taxonomic character because it is too much influenced by various factors. It would be interesting to study the chemical nature of the pigment.

The only possible conclusions are: very bright daylight always induces the production of red pigment in the strains, independent of their original colour. The red pigment also diffuses into the oatmeal agar. It occurs potentially in every strain mentioned in this paper. The development of pigment declines at decreasing pH. Cultures grown in the dark show more conspicuous pigmentation compared with those grown in the light, independently of the original colour. No obvious influence of Mg^{2+} and NO_3^- was observed.

HERBARIUM MATERIAL

Herbarium material of species of *Epicoccum* was kindly sent on loan by the directors of the Rijksherbaria of Groningen (**GRO**) and Leiden (**L**), the Botanischer Garten und Museum, Berlin-Dahlem (**B**), the Royal Botanic Gardens, Kew (**K**), and the Istituto ed Orto Botanico, University of Padua (**PAD**).

Type material of *E. nigrum* (Pl. 9, figs. 7–9) and of many other species was examined. More than once it proved that the specimen did not belong to *Epicoccum*. Generally the material was not sufficient to allow measurements of more than a few spores of every specimen. No real differences in the structure of the spores of the different species have been observed. Even in preparations of the 'species' described as having continuous spores, pluricellular conidia were observed. Often spores were noted which were much smaller or larger than the sizes mentioned in the diagnoses. In every collection examined spores with a basal cell were observed.

Thus the study of herbarium type and authentic material strengthens the opinion that the many unsatisfactorily described species are not different from *E. nigrum.*

Specimens examined

E. nigrum Link, type, **B**; **L** Roumeguère, Fungi gallici exsiccati 1155; Ellis, North American Fungi 1228; **K** fol. 3480.

E. caricicolum Schw., type, **K** 287.

E. dispersum Ces. & Mont., type, **K**, **L** Rabenhorst, Fungi europaei 472.

E. eucalypti Henn., type, **B** 388.

E. intermedium Allesch., lectotype, **K**, **L** Rabenhorst-Parschke, Fungi europaei et extra europaei 4300; **L** Sydow, Mycotheca germanica 850, 2999; 2939 under the name *Clathrococcum intermedium* Keissler.

E. javanicum Koord., type, **B**.

E. ligustri Henn., type, **B** 398.

E. menispermi Pass., type, **B**, **L** Rabenhorst, Fungi europaei 2463, **L** 749.

E. micropus Corda, type, **K** fol. 3492, *Epicoccum* no more present; Ascot 1867; **GRO** leg. Beins 1899.

E. microscopicum Henn., type, **B** 393.

E. neglectum Desm., type, **K**, **PAD** 540; **K** 2478; **L** Sydow, Mycotheca germanica 447, 2800; Rabenhorst, Fungi europaei 857; Kryptogamae exsiccatae 2130; Ellis, North American Fungi 1227; **GRO** fol. 88.

f. *caricis*, **L** Roumeguère, Fungi gallici exsiccati 1999.

f. *menispermi canadensis*, type, **GRO** Saccardo, Mycotheca veneta 1241.

f. *scirpi*, **L** Roumeguère, Fungi gallici exsiccati 1773.

f. *stromate rufescente*, **L** Saccardo, Mycotheca italica 1745.

f. *vitis vinifera*, **L** de Thümen, Mycotheca universalis 1496.

E. pandani Henn., type, **B** 278.

E. polygonati Henn. & Shir., type, **B** 148.

E. purpurascens Ehrenb., type, **B**; **L** de Thümen, Mycotheca universalis 1879; Saccardo, Mycotheca italica 1197; Rabenhorst, Herbarium Mycologicum Ed. 2: 785; Otto Jaap, Fungi selecti exsiccati 600; Allescher & Schnabl, Fungi bavarici 299; Roumeguère, Fungi gallici exsiccati 945; Ellis & Everhart, North American Fungi sec. ser. 2398, 2398b; **PAD** 684; Saccardo, Mycotheca italica 1197; de Thümen, Mycotheca universalis 1879; Allescher & Schnabl, Fungi bavarici 299; **GRO** 88. *E. commune* Corda var. *bulbicola*, type, **L** Roumeguère, Fungi gallici exsiccati 3295.

f. *lacrymae* **K** Roumeguère, Fungi selecti exsiccati 6016.

E. reticulatum Cooke, type, **K** fol. 3506.

E. rhodotypi Henn., type, **B** Sydow, Mycotheca Marchica 4895.

E. triticae Henn., type, **B** 259.

E. maculatum Cooke, type, **K**, fol. 3387, is *Papularia sphaerosperma* (Pers.) v. Höhn.

E. pallescens Berk., type, **K** fol. 3498. Sporodochia with setae, conidia smooth, pale, most probably not *Epicoccum.*

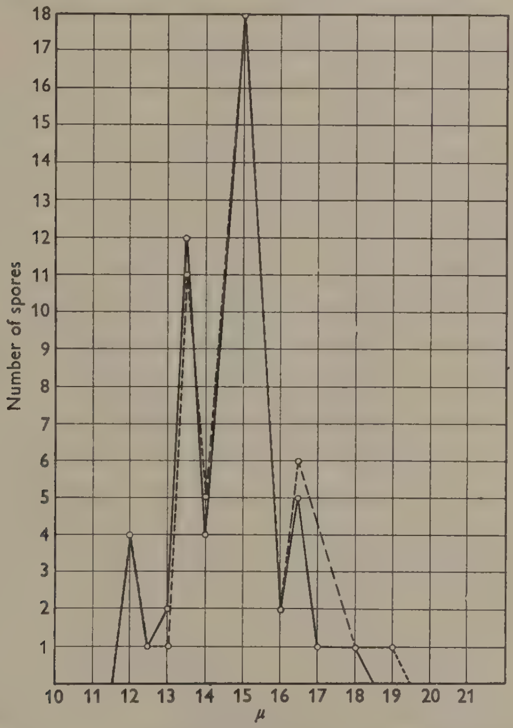
E. simplex Berk. & Curt., type, **K** 287, 3500 is *Papularia sphaerosperma.*
E. tiliae nomen nudum, type, **B**, *Epicoccum* not present.
E. atro-sanguineum Wallr., **GRO** 234.
E. atrum Link, **L** Roumeguère, Fungi gallici exsiccati 944. The material did not provide a ripe sporodochium.
E. cauvinianum Mont., **K** fol. 3483.
E. diversisporum Preuss, **GRO** 2215, **K** 305.
E. duriaeanum Mont., **GRO** 2111, **K** 3041, **L** Rabenhorst, Fungi europaei 471, Ellis, North American Fungi 638.
E. effusum Fuckel, **GRO** 1529.
E. equiseti Berk., **GRO** 237.
Clathrococcum granulatum Penz. = *E. granulatum* Penz., **K** fol. 3484.
E. granulatum Penz., **L** Sydow, Mycotheca germanica 2049.
f. *sorghi*, **K** fol. 3484.
E. herbarum Corda, **K** 3297b.
E. laeve Corda, **L** Herbarium Hasskarl 226.
E. lineolatum Desm., **L** Roumeguère, Fungi gallici exsiccati 2482. *Epicoccum* not present.
E. maritimum Suth., **K** 4988.
E. neglectum Oudem., **L** 347a is not *Epicoccum.*
E. platani Fuckel, **L** Herbarium Hasskarl 227.
E. scabrum Corda, **K** fol. 3491; **L** Rabenhorst, Fungi europaei 690; Ellis & Everhart, North American Fungi sec. ser. 2397; **GRO** fol. 88.
E. sphaeroides Corda, **L** Herbarium Hasskarl 225; **GRO** 231.
E. sphaerospermum nomen nudum, **PAD** Ravenel, Fungi caroliniani exsiccati is *Papularia sphaerosperma*; **K** 2608.
E. usneae Anzi, **L** Otto Jaap, Fungi selecti exsiccati 74.
E. versicolor Rabenh., **L** Rabenhorst-Winter, Fungi europaei 2892; **PAD.**
E. vulgare Corda, **L, PAD** Saccardo, Mycotheca italica 596; **K** Roumeguère, Fungi gallici exsiccati 3497, in bad condition; **PAD** Bail Herbarium mycologicum typicum and 8 other specimens; **GRO** 2 specimens.
f. *spiraeae* **L, PAD** Saccardo, Mycotheca italica 1597.

MEASUREMENTS OF SPORES OF THE STRAINS CULTURED

As hitherto no dependable character of specific value in the 70 strains had been found, it seemed necessary to measure spores of the 47 well-fructifying strains. The other 23 strains produced no spores in culture or not enough to be used for measurements. For comparison, only ripe conidia with a ring-shaped basal cell (Text-fig. 1 *g*) were measured. Moreover, both apex and base of the spore must lie in the same plane of vision, so that the true length could be measured.

Fulfilling these precautions, only a rather small number of measurable spores were available in a normal preparation and therefore the measurements were limited to 50 conidia of each strain. As soon as a transfer began to fructify, the spores were measured (first examination). Often, after some time in culture, the measurements were repeated (second examination). Exceptionally a third was made. The isolates were grown on sterilized *Lupinus* stems.

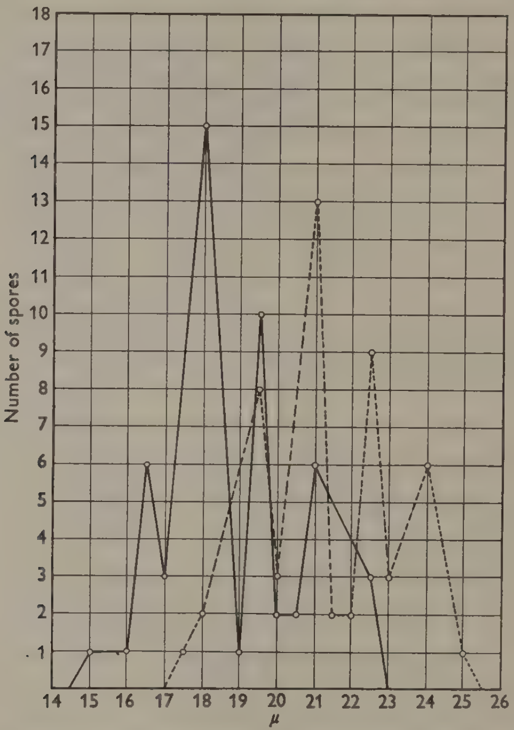
Length and width were set out in curves. Every curve for length as well as for width shows more than one peak. Text-figs. 2 and 3 show typical curves. Because every curve appeared to have a number of peaks, it seemed necessary to try and see if these peaks would disappear in curves prepared from 200 measurements. A single spore culture of the strain *Cooke* 1842 (marked *Cooke* 1842a) was used to measure 200 spores. The curves so obtained also have several peaks (Text-fig. 4) and they are comparable with those of curves prepared from fifty measurements of the other strains. Therefore, it seemed sufficient to measure 50 spores of every strain.



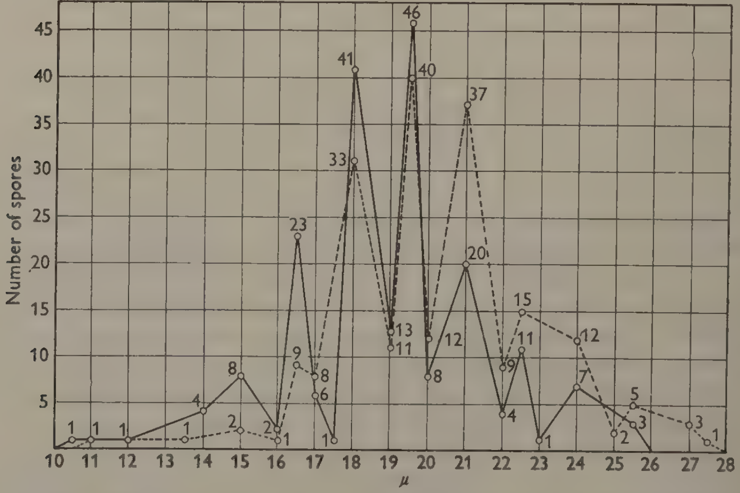
Text-fig. 2. Dimensions of 50 spores of *E. nigrum* strain *Orie.*
—— length; ---- width

It is possible to range the curves by considering the most prominent three peaks of every curve. The head peak of the spore length marked with ▲ is flanked by two next lower peaks marked with △. The head peak of the spore width marked with ■ is flanked by the two next lower peaks marked with □. Table 2 gives the general survey of the peaks of the curves. The peaks of length and width coincide when the spores are preponderantly round (Text-fig. 2 and Table 2, nos. 2, 7, 9, 11, 12, 13, 14). More often most of the spores are broader than long and then the peaks of the width lie more to the right (Text-fig. 3 and Table 2, nos. 8, 23, 24, 25, 26, 27, 28, 30, 31, 35, 36, 37, 38, 39, 43, 45). The curves of the strain *Cooke* 2119 (Table 2, no. 32) show that most of the spores in this strain are longer than broad.

The variability of the spore sizes appears also from the measurements in two different preparations of the same culture as well as from examinations of the same strain at different times of cultivation. Sometimes it



Text-fig. 3. Dimensions of 50 spores of *E. nigrum* strain *Lobry de Bruyn*, no. 2.



Text-fig. 4. Dimensions of 200 spores of *E. nigrum* monospore strain *Cooke* 1842a.

Table 2

Spore ranges (μ)	Highest peaks of length and width (μ)	Number and name of the strain	Peaks of length and width (μ)																								
			9	10		11	12	13		14	15	16		17	18	19	20	21	22	23	24	25	26	27	28	29	30
7–17×9–19·5	12×12	1 *Epicoccum F*419			△		▲				△																
			□				■				□																
9–15×9–16	12×12	2 *E. oryzae* 2			△		▲		△																		
					□		■		□																		
9–19×9–19	12×15	3 *Baruah*			△		▲				△																
							□		□		■																
9–17×10–18	12×12	4 *E. purpurascens* 1st exam.			△		▲				△																
					□		■	□			□																
12–18×13–19·5	15×15	2nd exam.					△		△		▲																
											■		□		□												
9–18×10–18·5	15×15	5 *de Vries Mulder* 1st exam. } same tube					△		△		▲																
									□		■		□														
10·5–17×11–18	13·5×15	2nd exam. }					△		▲		△																
									□		■	□															
9–16×9–19·5	12×13·5	3rd exam.					▲		△		△																
									■		□	□															
10·5–16·5×12–19·5	13·5×15	6 *Ordman C* 1st exam.					△		▲		△																
									□		■				□												
12–19×12–21	15×15	2nd exam.							△	△	▲																
									□		■		□														
10·5–18×10·5–18	13·5×13·5	7 *Becker* 1					△		▲		△																
							□		■		□																
10–18×12–19·5	13·5×15	8 *Quintanilha* 47					△		▲		△																
									□		■		□														
10·5–19·5×10·5–18	15×15	9 *van Vloten*					△		△		▲																
							□		□		■																
11–18×10–18	15×15	10 *Lobry de Bruyn* 3					△		△		▲																
							□				■			□													
10·5–19·5×12·5–21	15×15	11 *Nicot*							△		▲		△														
									□		■		□														
11–17×11–18	15×15	12 *Irtel* 1st exam.							△		▲		△														
									□		■		□														
12–20×13–19·5	15×15	2nd exam.							△		▲		△														
									□		■		□														

Table 2 (*cont.*)

Spore ranges (μ)	Highest peaks of length and width (μ)	Number and name of the strain	Peaks of length and width (μ)																										
			9	10	11	12	13		14	15	16		17	18	19		20	21	22	23	24	25	26	27	28	29	30
10·5–18 × 10·5–19	15 × 15	13 *Mansens*	·	·	·	·	·	△	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·	□	·	■	·	□	·	·	·		·	·	·	·	·	·	·	·	·	·	·
12–18 × 12–19	15 × 15	14 *Orie*	·	·	·	·	·	△	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·	□	·	■	·	□	·	·	·		·	·	·	·	·	·	·	·	·	·	·
10·5–18 × 12–19	15 × 15	15 *Cooke* 2040	·	·	·	·	·	▲	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·	□	·	□	·		·	·	·	·	·	·	·	·	·	·	·
13–19·5 × 13–21	15 × 15	16 *Mulder* 21	·	·	·	·	·		·	▲	·	△	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·	□	·	□	·		·	·	·	·	·	·	·	·	·	·	·
9–22·5 × 9–22·5	15 × 15	17 *Leiden*	·	·	·	·	·		·	▲	△		·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·	□	·	□	·		·	·	·	·	·	·	·	·	·	·	·
10·5–21 × 13·5–22·5	15 × 15	18 *New Guinea*	·	·	·	·	·	△	·	▲	·		·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·		□	·	·		·	□	·	·	·	·	·	·	·	·	·
12–27 × 12–25·5	15 × 15	1st exam. 19 *Portugal C*	·	·	·	·	·	△	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·	□	·	□	·		·	·	·	·	·	·	·	·	·	·	·
13·5–22 × 13·5–24	15 × 18	2nd exam.	·	·	·	·	·	△	·	▲	·		△	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·		·	■	·	□	·	·	·	·	·	·	·	·	·	·	·
10·5–24 × 12–27	18 × 18	1st exam. 20 *Küster* 5-I-3	·	·	·	·	·		·	▲	·	△	·	▲	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·	□	·	■	·		·	·	·	·	·	·	·	·	·	·	·
12–20 × 11–19	15 × 15	2nd exam.	·	·	·	·	·		·	▲	·	△	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·	□	·	■	·		·	□	·		·	·	·	·	·	·	·	·	·	·	·
11·5–27 × 12–39	18 × 19·5	1st exam. 21 *Bergen* 44	·	·	·	·	·	△	·	·	·	△	·	▲	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·		·	□	·	■	·	·	·	·	·	·	·	·	·	·	·
12–20 × 12–19·5	15 × 15	2nd exam.	·	·	·	·	·	△	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·	□	·	■	·	□	·	·	·		·	·	·	·	·	·	·	·	·	·	·
12–20 × 13·5–21	16·5 × 18	1st exam. 22 *Red currant*	·	·	·	·	·		·	△	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·		·	■	·		·	□	·	·	·	·	·	·	·	·	·
10·5–21 × 10–22·5	15 × 15	2nd exam.	·	·	·	·	·		·	▲	·	△	·	·	△		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	■	·	□	·	□	·		·	·	·	·	·	·	·	·	·	·	·
12–18 × 13–19·5	15 × 16·5	23 *Cooke* 1842	·	·	·	·	·	▲	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·	□	·	□	·	■		·	·		·	·	·	·	·	·	·	·	·	·	·
13–18 × 15–22	15 × 16·5	24 *Cooke* 724	·	·	·	·	·	△	·	▲	·	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·	■	·	□	·		·	·	·	·	·	·	·	·	·	·	·

Table 2 (*cont.*)

Spore ranges (μ)	Highest peak of length and width (μ)	Number and name of the strain	Peaks of length and width (μ) 9	10	11	12	13		14	15	16		17	18	19		20	21	22	23	24	25	26	27	28	29	30
12–2 × 13·5–21	15 × 16·5	25 *Mulder* 41	·	·	·	·	·		·	▲	·	△	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·	■	·	■	·		·	·	·	·	·	·	·	·	·	·	·
13–19·5 × 15–21	16·5 × 18	26 *Cooke* 2359	·	·	·	·	·		·	▲	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·	□	·	■	·	□	·	·	·	·	·	·	·	·	·	·	·
13·5–22·5 × 13·5–22·5	16·5 × 18	27 *Frico*	·	·	·	·	·		·	△	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·	□	·	■	·	□	·	·	·	·	·	·	·	·	·	·	·
12–27 × 12–27	15 × 18	1st exam. } same	·	·	·	·	·		·	▲	△	△	·	·	·		·	·	·	·	·	·	·	·	·	·	·
		28 *Verona*	·	·	·	·	·		·	□	·	■	·	·	·		·	□	·	·	·	·	·	·	·	·	·
13·5–22 × 14–23	16·5 × 18	2nd exam. } tube	·	·	·	·	·		·	△	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·	□	·	■	□		·	·	·	·	·	·	·	·	·	·	·
12–22·5 × 14–25	15 × 18	1st exam.	·	·	·	·	·		·	▲	·	△	·	△	·		·	·	·	·	·	·	·	·	·	·	·
		29 *E. granul. Heald*	·	·	·	·	·		·	·	·	□	·	■	□		·	·	·	·	·	·	·	·	·	·	·
12–19·5 × 14·5–22·5	15 × 15–16·5–17–21	2nd exam.	·	·	·	·	·	△	·	▲	·		△	·	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·		□	·	·		·	□	·	·	·	·	·	·	·	·	·
10–22·5 × 12–25·5	15 × 18	1st exam.	·	·	·	·	·		·	▲	·	△	·	△	·		·	·	·	·	·	·	·	·	·	·	·
		30 *Cajkovac*	·	·	·	·	·		·	·	·	□	·	■	·	□	·	·	·	·	·	·	·	·	·	·	·
14–20 × 15–22·5	18 × 19·5	2nd exam. } same	·	·	·	·	·		·	·	·	△	·	▲	·	△	·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·		·	□	·	■	·	□	·	·	·	·	·	·	·	·	·
14–22·5 × 15–23	16·5 × 17	3rd exam. } tube	·	·	·	·	·		·	△	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·	□	■	□	·		·	·	·	·	·	·	·	·	·	·	·
12–30 × 10–27	16·5 × 18	31 *de Area Lĕao*	·	·	·	·	·		·	△	·	▲	·	△	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·	□	·	■	·	□	·	·	·	·	·	·	·	·	·	·	·
13·5–27 × 15–27	18 × 16·5	32 *Cooke* 2119	·	·	·	·	·		·	△	·	△	·	▲	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	□	·	■	·	□	·		·	·	·	·	·	·	·	·	·	·	·
13·5–22 × 15–24	18 × 18	33 *Cooke* 2388	·	·	·	·	·		·	△	·	△	·	▲	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·		·	■	·	□	·	□	·	·	·	·	·	·	·	·	·
13·5–25·5 × 15–36	18 × 18	34 *Lupinus*	·	·	·	·	·		·	△	·	△	·	▲	·		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·		·	■	·	□	·	□	·	·	·	·	·	·	·	·	·
14–21 × 14–22	18 × 19·5	35 *Loire* 2	·	·	·	·	·		·	·	·	△	·	▲	△		·	·	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·		·	□	·	■	·	□	·	·	·	·	·	·	·	·	·
14–22·5 × 14–22·5	18 × 19·5	36 *Koelhuis*	·	·	·	·	·	△	·	·	·		·	▲	·		·	△	·	·	·	·	·	·	·	·	·
			·	·	·	·	·		·	·	·		·	□	·	■	·	□	·	·	·	·	·	·	·	·	·

Table 2 (*cont.*)

Spores ranges (μ)	Highest peaks of length and width (μ)	Number and name of the strain	Peaks of length and width (μ)																									
			9	10	11	12	13	14	15	16		17	18	19		20	21	22		23	24	25		26	27	28	29	30
15–21 × 16–22·5	18 × 19	37 *E. vulgare* 1st exam. ⎫ same tube									△		▲		△													
													□		■		□											
14·5–19·5 × 15·5–22·5	15 × 18	2nd exam. ⎭							▲			△	△															
												□	■			□												
17–24 × 15·5–27	19·5 × 22–22·5	3rd exam.											△		▲		△											
																	□	■	■									
15–25 × 16–24	18 × 19·5	38 *van Holder*									△		△		△													
													□		■				□									
13–25·5 × 15–30	18 × 21	39 *Espagne* 6							△		△		▲															
											□				□		■											
15–22·5 × 17·5–25	18 × 21	40 *Lobry de Bruyn* 2 1st exam.									△		▲		△													
															□		■		□									
15–22·5 × 16·5–23·5	19·5 × 19·5	2nd exam.											△		▲		△											
															■		□		□									
15–25·5 × 16·5–25·5	21 × 22·5	41 *Quintanilha* 66									△		△				▲											
															■				□		□							
17–27 × 17–25·5	21 × 21	42 *van Beverwijk*											△		△		▲											
													□				■		□									
14–33 × 16–36	21 × 22·5	43 *Portugal A*											△		△		▲											
																	□		■		□							
16·5–24 × 18–27	19·5 × 22	44 *Witkamp* 1st exam.											▲		▲		△											
															□				■		□							
15–24 × 15–27	21 × 21	2nd exam.								△			△				▲											
																	■		□		□							
16·5–25·5 × 19–30	21 × 22	45 *Portugal D*											△				▲		△									
																	□		■		□							
16·5–28·5 × 18–30	21 × 22·5	46 *Beach chair*											△		△		▲											
															□		■		■									
22·5–54 × 21–49	25·5 × 30	47 *J. Smit* 1st exam.																	△				▲					△
																			□				□					■
14–25 × 16·5–30·5	21 × 21	2nd exam.											△		△		▲											
													□				■				□							

appears that the spores have become larger (Table 2, nos. 4, 19, 28, 37), sometimes that they have decreased in size (Table 2, nos. 5, 21, 22).

The shape of the conidia also varies greatly; according to the curves of the strain *de Vries Mulder* (Table 2, no. 5) the spores at the time of the first examination were mostly globose, at the time of the second and third examinations, however, they seemed to be preponderantly broader than long.

The variability in the strains *Cajkovac* (Table 2, no. 30) and *J. Smit* (Table 2, no. 47) is obvious. In the first examination of no. 47 the head peaks for length and width lie at 25 and 30 μ, in the second examination they were at 21 μ.

From Table 2 it appears that the group of the first 8 strains has rather small spores (7–19 × 9–21 μ). The 7 strains at the end of the table form a group with large spores (14–54 × 13·5–49 μ). Each of these groups gradually merges into a middle group of 32 strains with intermediate spores. There is no distinct delimitation between these groups.

DISCUSSION

This study has led to the opinion that the development and the structure of the spore are the only constant factors in every strain and that none of the other characters can be considered as taxonomic characters. The numerous measurements show that the spore sizes vary at different times of examination and in different respects, not only in one and the same culture, but also during prolonged cultivation of the strain. It is clear that at the time of identification the different factors which influence the spore size cannot be known. No different, well established species can be distinguished in the 47 sporulating strains. Therefore the only logical course seems to be to class these strains as one species, *Epicoccum nigrum* Link.

The appearance of cultures of *Epicoccum*, even when not sporulating, is so typical that, in spite of the divergency in colour, they can be recognized as *Epicoccum*. Thus, the appearance of the other 23 strains made it most probable that they also belong to *Epicoccum*. Some of them sporulated scantily but lost this ability very soon. The few spores obtained showed that these strains too did not differ from *E. nigrum*. Others are derivatives of typae isolates or authentic material which have lost the ability of sporulting.

A critical study of the diagnoses and of the herbarium material, especially that of the different types and the authentic material, warrant the opinion that most of the described species are identical with *E. nigrum* and that others do not belong to the genus *Epicoccum* after the revision of this genus.

The conclusion is that, up till now, no species other than *E. nigrum* has been encountered.

EXCLUDED OR DOUBTFUL SPECIES

Mason (1927) eliminated *E. hyalopes* Miyaki and *E. levisporum* Pat. 'both very closely related if not identical with *Nigrospora oryzae* (B. & Br.) Petch.'; and Mason (1933) also declared that *E. simplex* Berk. & Curt. issued in Ravenel as *E. sphaerospermum* (a nomen nudum) belongs to the *Papularia sphaerosperma* group.

After studying the type material, the description and figures, I am of opinion that *E. maculatum* Cooke, type, **K** fol. 3387, *E. theobromae* Petch, in *Ann. R. bot. Gdns. Peradeniya*, **19**, 328, 1924, and *E. magnoliae* Togn. in Saccardo, *Syll. Fung.* **11**, 654, 1895, belong to the *Papularia* group.

E. coniferarum Petr., in *Hedwigia*, **74**, 74, 1935, with slimy spores, 5–7·5 × 4·5–7 μ, cannot be *Epicoccum* but is probably also *Papularia*.

E. magnoliae Togn. var. *verrucosum* Bong., in *Boll. Lab. sper. Fitopat. Torino*, N.S., **18**, 55, 1942, may be *E. nigrum*.

E. neglectum Oudem. **L** 3474a, 1870, is no *Epicoccum*.

E. pallescens Berk., type, **K** fol. 3498 has sporodochia with setae and the spores always have something as a pedicel, sometimes with appendix; they are smooth, very pallid, almost hyaline. The species does not resemble an *Epicoccum*.

E. cocus Stevens, in the *Philip. Agric.* **21**, 80, 1932–33 is, according to the description and the figure, no *Epicoccum*.

E. echinatum Pegl., in Saccardo, *Syll. Fung.* **11**, 654, 1895, has been described with long spines (12–14 μ) on the spores and H. & P. Sydow changed it to *Petrakia echinata* (Pegl.) Syd., in *Ann. mycol., Berl.*, **11**, 407, 1913.

E. davidsonii Rostr., in *Bot. Tidsskr.* **25**, 324, 1903, has been described with pedicels varying from 25 to 30 μ, which is abnormally long. Doubtful synonymy with *E. nigrum*.

E. quercinum Berk. & Curt. seems to be unpublished. On the material from Kew Herbarium no *Epicoccum* has been found.

Species incorrectly described with only unicellular spores are: *E. asterinum* Pat., *E. atrosanguineum* Wallr., *E. chrysanthemi* du Pless., *E. plagiochilae* Racov., *E. sinense* Petr., and *E. torquens* Mass.

Species reported to occur on living plants

Author	Fungus	Host
Nicolas & Dop (1926)	*E. asperulum*	*Citrus aurantium*
	E. atrosanguineum	*Acorus calamus*
	E. vulgare	*Euphorbia prunifolia*
	E. purpurascens	*Holcus mollis*
	E. nigrum	*Cucurbita ficifolia*
Koorders (1907)	*E. javanicum*, a weak parasite	*Ficus quercifolia*
Zeretelli (1929)	*E. neglectum*, an insignificant parasite	*Vitis vinifera*
Zaprometoff (1931)	*E. neglectum*, a significant parasite	*Corchorus capsularis*
Ito & Iwadare (1932)	*E. oryzae* and *E. neglectum*, positive results with infection experiments and cause of a complete loss of germinative capacity	*Oryza sativa*
Hopkins (1932)	*E. purpurascens*, a virulent wound parasite	Cotton
Peters & Schwartz (1912)	*E. purpurascens*	Tobacco
Massee (1906)	*E. torquens*	Capsules of *Weisia viridula*
Hennings & Shirai (1900)	*E. polygonati*	*Polygonatum officinalis*
Ruehle (1931)	*E. granulatum*	Apple
Patouillard (1912)	*E. asterinum*	*Yucca elephantipes*
Săvulescu (1942)	*E. yuccae*	*Yucca filamentosa*
Petrak (1947)	*E. sinense*	*Astralagus sinicus*
Racovitza (1944)	*E plagiochilae*	*Plagiochila asplenioides*
Du Plessis (1933)	*E chrysanthemi*	*Chrysanthemum* sp.

The relationship between the genera *Epicoccum* and *Cerebella* has now to be considered.

Cesati (1851) established the genus *Cerebella* (type *C. andropogonis*). He described the stromata of this species as convoluted, occurring between the glumes of the spikelets of *Andropogon ischaemon* L., in Northern Italy, consisting of whitish cells and abundantly producing not very small, dark spores. This description is unsatisfactory, nothing being said about the structure of the spores and their measurements.

Langdon (1942) pointed to the confusion existing about *Cerebella*. During many years the fungus was regarded as a true smut. He made it evident that *Cerebella* is a saprophyte on the honey-dew of ergot and provides a natural control of ergot. (I mean that therefore *Cerebella* is more or less parasitic on *Claviceps*.) *Cerebella* is a good field indicator of the presence of ergot.

Langdon (1955), in his monograph of the genus *Cerebella*, pointed to the fact that Mattirolo (1897) suggested that the genera *Cerebella* and *Epicoccum* should be united because they agree in so many respects. However, the pluricellularity of the *Epicoccum* spore was still a doubtful point in 1897.

Langdon (1955) did not unite the two genera either, because of the unpublished information by S. J. Hughes on the development of *E. purpurascens*. I cite from Langdon: 'In this species [*E. purpurascens*] the spore is an aleuriospore and pluricellular. A constant feature is a small basal cell just above the stalk cell. In *C. andropogonis* there is no small basal cell constantly developed and the first septum laid down may be a vertical one.'

In the Centraalbureau 4 strains of *C. andropogonis* Ces. are cultured. In every case where the development of the aleuriospores in these strains has been observed, the first wall in a sporiferous hypha is always a transverse wall cutting off the spore initial. The second wall is also a cross-wall by which the basal cell is formed.

It is considered that there is no real difference between the two genera and that *Cerebella* should be regarded as a synonym of *Epicoccum*. Pl. 9, fig. 11 shows a young sporodochium and spores in pure culture.

The second species of *Epicoccum* is therefore *Cerebella andropogonis* Ces. Its spores are without any doubt pluricellular and more constricted at the septa than those of *E. nigrum*. They are less verrucose than the spores of *E. nigrum* and the basal cell is always conical or cylindrical. In nature its sporodochia are cerebriform but in culture they become less convoluted and more globose or subglobose.

EPICOCCUM Link, in *Mag. Ges. Naturf. Fr. Berl.* **7**, 32, 1816

Sporodochia with hyaline conidiophores producing a single dark pluricellular aleuriospore in which first the basal cell, which may be shed, is developed. Stromata hemispherical, spherical or cerebriform. Type: *Epicoccum nigrum* Link.

(1) *Epicoccum nigrum* Link, in *Mag. Ges. naturf. Fr. Berl.* **7**, 32, 1816.

Synonyms according to Saccardo and to the checked type material.

E. caricicolum Schw., in *Syn. Amer. bor.* f. 3039, no. 287.
E. dispersum Ces. & Mont., in *Bot. Z.*, **20**, 199, 1862.
E. eucalypti Henn., in *Hedwigia*, **41**, 311, 1902.
E. granulatum Penz., in Fung. Agrum., *Michaelia*, **2**, 487, 1882.
E. granulatum Penz. var. *microspora* Gonz. Frag., *Bol. Soc. esp. Hist. nat.* **23**, 526, 1923.
E. heterochroum ad int. C.B.S.
E. intermedium Allesch., in *Ber. bayer. bot. Ges.* **4**, 39, 1896.
E. javanicum Koord., in *Verh. Akad. Wet. Amst.* 2e Sect., **13**, 4, 249 fig. 60, 1907.
E. ligustri Henn., in *Hedwigia*, **41**, 311, 1902.
E. menispermi Pass., in *Hedwigia*, **17**, 172, 1878.
E. microscopicum Henn., in *Hedwigia*, **41**, 311, 1902.
E. neglectum Desm., in *Ann. Sci. nat.* 2e ser., **17**, 95, 1842.
E. oryzae Ito & Iwad., in *Rep. Hokkaido agric. Exp. Sta.* **31**, 1, 1934.
E. pandani Henn., in *Hedwigia*, **41**, 118, 1902.
E. polygonati Henn. & Shir., in *Engler's Jahrb.* **29**, 153, 1900.
E. purpurascens Ehrenb. ex Schlecht, in *Flora Berol.* **2**, 136, 1824.
E. reticulatum Cooke, in Saccardo, *Syll. Fung.* **4**, 741, 1886.
E. rhodotypi Henn., in *Not. bot. Gart. Berl.*, **2**, n. 20, 383, 1899.
E. tritici Henn., in *Hedwigia*, **43**, 146, 1904.
E. versicolor Rabenh., in *Dtsch. Krypt. Fl.* **1**, 54, 1884.
E. vulgare Corda et diff. formae, in *Icon. Fung.* **1**, 5, 1837.

Synonyms according to conclusions from various authors, from checked herbarium material and from critical study of descriptions and figures.

E. agyrioides Corda, in *Icon. Fung.* **1**, 5 (fig. 89), 1837.
E. agyrioides Corda var. *pineum* Sacc., in *Michaelia*, **2**, 366, 1880.
E. angulosum Penz. & Sacc., in *Malpighia*, **15**, 258, 1901.
E. asperulum Otth, in *Mitt. naturf. Ges. Bern*, no. 62, 1868.
E. asterinum Patouil., in *Bull. Soc. mycol. Fr.* **28**, 143, 1912.
E. atro-sanguineum Wallr., in *Fl. Crypt. Germ.* **2**, 234, 1833.
E. cauvinianum Mont. **K.** 3483.
E. chrysanthemi du Plessis, in *Ann. Univ. Stellenbosch*, **11** A2, 11, 1933.
E. compactum Berk. & Curt., in Fungi Cub., *J. Linn. Soc. Lond.* **10**, part 2 n, 623, 1869.
E. diversisporum Preuss, in *Linnaea*, **25**, 740, 1852.
E. diversisporum Preuss, var. *pseudoplatani* Allesch., in *Hedwigia*, **34**, 290, 1890.
E. duriaeanum Mont., in *Ann. Sci. nat.* 3e ser., **11**, 38, 1849.
E. effusum Fuck., in *Symb. myc.* 373, 1869.
E. equiseti Berk., in *Outlines*, 341, 1860.
E. equiseti-limosi Allesch., in *Ber. bayer. bot. Ges.* **5**, 22, 1897.
E. fructigenum McAlp., in *Fungus Diseases of Stone Fruit Trees in Australia*, 1902.
E. herbarum Corda, in *Icon. Fung.* **1**, 5 (fig. 88), 1837.
E. humicola (Buch.) Sacc., in *Syll. Fung.* **25**, 985, 1931.
E. laeve Corda, in *Icon. Fung.* **1**, 5 (fig. 92), 1837.
E. lineolatum Desm., in *Not. Ann. Sc. nat.* **13**, sér. Bot., 6, 84, 1846.
E. majus Rostr., in Norske Ascom. *Skr. videnskSelsk., Christ.* **1**, 40, 1904.
E. malinvernianum Ces. & de Not., in *Erb. crittog. Ital.* Ser. 1, n. 789, 1862.
E. maritinum Suth., in *New Phytol.* **15**, 47, 1916.
E. mezzetti Goid., in *Boll. Staz. Pat. veg. Roma*, N.S., **17**, 443, 1937.
E. micropus Corda, in *Icon. Fung.* **3**, 32 (fig. 82), 1839.
E. negundinis Otth, in *Mitt. naturf. Ges. Bern*, no. 111, 1870.
E. panici Gz. Frag., in *Bol. Soc. esp. Hist. nat.* **18**, 85, 1918.
E. plagiochilae Racov., in *Bull. Acad. Roum.* (Sect. Sci.), **27**, 1, 1944.
E. platani Fuck., in *Enum. Fung. Nass.* p. 33 (fig. 25), 1860.
E. scabrum Corda, in *Icon. Fung.* **1**, 5 (fig. 91), 1837.
E. sinense Petrak, in *Medd. Goteb. bot. Trädg.* **17**, 162, 1947.

E. sphaerioides Corda, in *Icon. Fung.* **1**, 5 (fig. 93), 1837.
E. sulcatum Delac., in *Bull. Soc. mycol. Fr.* **17**, 192 (fig. 4), 1892.
E. torquens Mass., in *Torreya*, **6**, 48, 1906.
E. usneae Anzi, in *Atti Soc. ital. Sci. nat.* **11**, 181, 1868.
E. yuccae Săvul., in *Bull. Acad. Roum.* (Sect. Sci.), **25**, 28, 1942.

Sporodochia powdery, brownish to black, scattered or aggregated, mostly on distinct, variably coloured spots on decaying, seldom on living parts of various plants; on paper or textile, up to 2000 μ wide. Stromata hemispherical to spherical, conidiophores claviform, 0–2 septate, smooth, hyaline, up to 9 × 6 μ, producing a single dark aleuriospore. Mature conidia golden brown, brown, olivaceous or black, up to 15-celled, globose, subglobose, pyriform, sometimes of irregular, angular shape, irregularly septate, covered by a variably thick areolate epispore, verrucose, especially at the distal end; at the base with a scar surrounded by a ring-shaped basal cell, lighter in colour and less verrucose than the other part: 7–65 × 6–54 μ. The spores germinate with many germ tubes. Unripe conidia with a conical basal cell, sometimes also with a hyaline collar being a part of the wall of the stalk cell.

In pure culture the mycelium may easily be recognized by the colour, which varies from pink to red, purple, yellow, or olivaceous green to brown. Different colours often in one colony.

World wide, omnivorous, also isolated from the soil, the air, insects, human skin and sputum, textile; saprophytic or slightly parasitic.

(2) **Epicoccum andropogonis** (Ces.) comb.nov.

Basionym: *Cerebella andropogonis* Cesati, apud Rabenhorst, in *Bot. Ztg.* **9**, 669, 1851.

Sporodochia on Gramineae cerebriform, in pure culture hemispherical to spherical; conidiophores claviform, 0–2 septate, smooth, hyaline, up to 9 × 6 μ, producing a single dark aleuriospore, 2–many celled, irregularly septate, constricted at the septa, up to 30 × 27 μ, but often smaller and of irregular shape, at the base a conical or cylindrical basal cell; epispore usually verruculose, but sometimes smooth. The spores germinate with one or more germ tubes. In pure culture the colour of the mycelium varies from greyish white to dark olivaceous, sometimes with a tinge of red.

World wide, occurring on Gramineae infected with *Claviceps* spp. and living on the honey-dew secretions of this fungus, inhibiting the development of its sclerotia.

Living cultures of the well-sporulating strains of both species are maintained at the C.B.S. and will be sent to the Commonwealth Mycological Institute, Kew, England. Dried cultures will be deposited in the Rijksherbarium, Leiden, The Netherlands, and in the Herbarium of the Royal Botanic Gardens, Kew, England.

I am greatly indebted to Miss A. L. van Beverwijk and Dr S. J. Hughes for their most valuable suggestions, to Mr P. den Houter who prepared the microtome sections and most of the photographs and to Ir. R. C. Schol who drew the graphs.

REFERENCES

BUCHANAN, R. E. (1911). A new species of *Thyrococcum*. *Mycologia*, **3**, 3, plates 34–35.

CESATI, V. (1851). Apud Rubenhorst, in *Bot. Ztg.* **9**, 669.

DU PLESSIS, S. J. (1933). Drie nuwe siektes van blomtuinplante. *Ann. Univ. Stellenbosch*, **11**, Ser. A, 2.

ELLIS, J. J. (1956). A note on *Epicoccum*. *Proc. Iowa Acad. Sci.* **63**, 307.

GOIDÀNICH, G. (1937). Studi sulla microflora fungina della pasta di legno destinata alla fabbricazione della carte (II parte). *Boll. Staz. Pat. veg. Roma*, N.S., **17**, 443.

HENNINGS, P. & SHIRAI, M. (1900). *Englers Jb.* **29**, 153.

HÖHNEL, F. VON (1910). *Rabenhorst Kryptogamen Flora*, **9**, 595.

HOPKINS, J. C. F. (1932). Some diseases of cotton in Southern Rhodesia. *Emp. Cott. Gr. Rev.* **9**, no. 2, 109–118.

ITO, S. & IWADARE, S. (1932). Studies on the red blotch of rice grains. *Rep. Hokkaido agric. Exp. Sta.* **31**, 1–84. [*Rev. appl. Mycol.* **13**, 538, 1934.]

KOORDERS, S. H. (1907). Botanische Untersuchungen über einige in Java vorkommende Pilze, u.s.w. *Verh. Akad. Wet. Amst.* 2e Sect., **13**, 4, 249 (fig. 60).

LANGDON, R. F. N. (1942). The genus *Cerebella* Cesati—its biologic status and use. *Phytopathology*, **32**, 613.

LANGDON, R. F. N. (1955). The genus *Cerebella*. *Mycol. Pap.* 61.

LINDAU, G. (1910). *Rabenhorst Kryptogamen Flora*, **9**, 594.

LINK, H. F. (1816). Observationes in Ordines plantarum naturales 3. *Mag. Ges. naturf. Fr. Berl.* **7**, 32 (tab. 1, fig. 5).

MASON, E. W. (1927). On species of the genus *Nigrospora* Zimmermann recorded on Monocotyledons. *Trans. Brit. mycol. Soc.* **12**, 2–3, 152.

MASON, E. W. (1933). *Annotated account of fungi received at the Imperial Mycological Institute*, List 2, Fasc. 2, 19.

MASSEE, G. (1906). A fungus parasitic on a moss. *Torreya*, **6**, 1–5.

MATTIROLO, O. (1897). Il genere *Cerebella* di Vincenzo Cesati. *Mem. R. Accad. Bologna*, 5 Ser., **6**, 663–684.

MOREAU, C. & M. (1951). Sur quelques Hyphomycetes. *Bull. Soc. linn. Normandie*, 9e Ser., **6**, 79.

NAUMANN, C. W. (1912). *Epicoccum purpurascens* und die Bedingungen für seine Pigmentbildung. *Hedwigia*, **51**, 135.

NICOLAS, G. & DOP, P. (1926). Un parasite du Melon de Malabar. *Bull. Soc. mycol. Fr.* **42**, 194.

PATOUILLARD, N. (1912). Quelques champignons du Costa-Rica. *Bull. Soc. mycol. Fr.* **28**, 143.

PETERS, L. & SCHWARTZ, M. (1912). Krankheiten und Beschädigungen des Tabaks. *Mitt. Kais. biol. Anst. Land- Forstwirt.* **13**, 54.

PETRAK, F. (1947). Plantae Sinenses 42: Micromycetes. *Medd. Göteb. Bot.* **17**, 162.

RACOVITZA, A. (1944). *Epicoccum plagiochilae* sp.nov. champignon parasite du *Plagiochila asplenioides* var. *major* Ness. *Bull. Accad. Roum.* (Sect. sci.), **17**, 1.

RUEHLE, G. D. (1931). New apple-rot fungi from Washington. *Phytopathology*, **21**, 1141.

SACCARDO, P. A. (1886). *Syll. Fung.* **4**, 736.

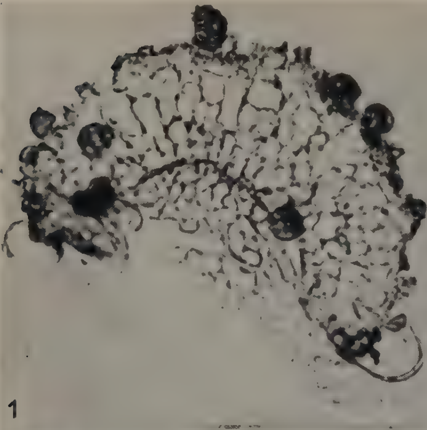
SACCARDO, P. A. (1931). *Syll. Fung.* **25**, 985.

SĂVULESCU, T. (1942). Einige neue Micromyceten. *Bull. Accad. Roum.* (Sect. sci.), **25**, 28.

WAKEFIELD, E. & BISBY, G. R. (1941). List of Hyphomycetes recorded for Britain. *Trans. Brit. mycol. Soc.* **25**, 99.

ZAPROMETOFF, N. G. (1931). Diseases of new bast-producing cultures in the region of Tashkent. In *Furtherance of the New Fibre*. Moscow.

ZERETELLI, L. J. (1929). Fungi collected on the vine in the district of the Sakaro. Exp. Sta. of Viticulture and Wine making. *Sci. Pap. appl. Sect. Tiflis bot. Gdn.*



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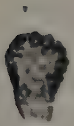

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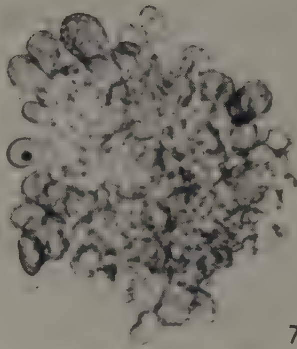

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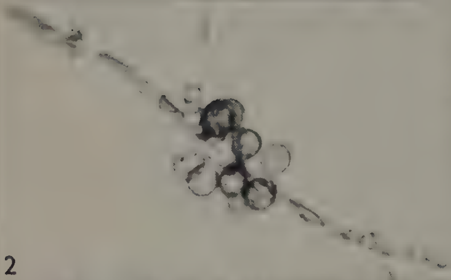

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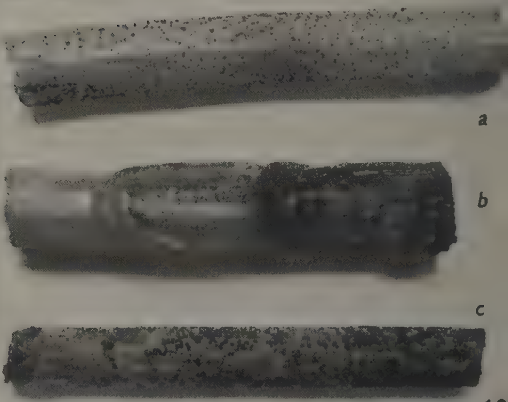

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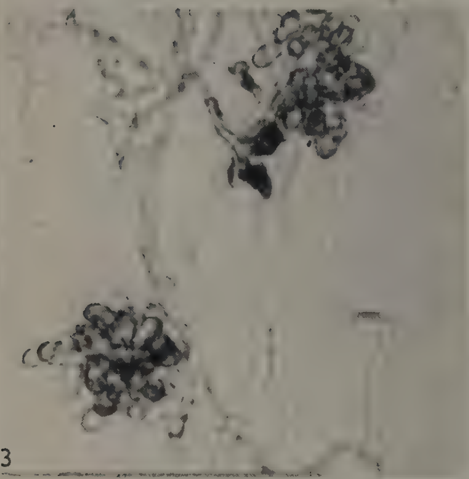

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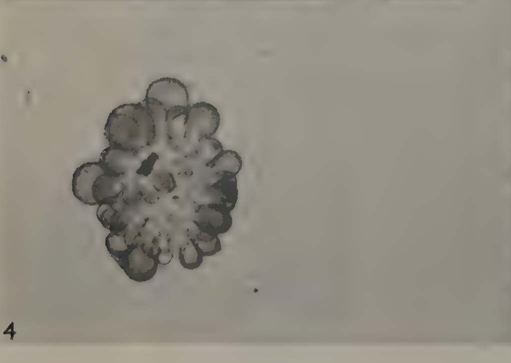

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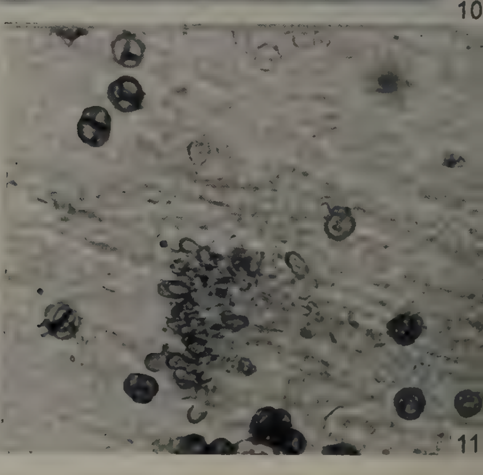

11

EXPLANATION OF PLATE 9

Fig. 1. Section of a sporodochium showing the pseudoparenchymatous stroma and the different stages of maturity of the spores. × 400.
Fig. 2. Spore development at both sides of a hypha. × 200.
Fig. 3. Very young sporodochia. × 300.
Fig. 4. Ripe sporodochium with spores in different stages of maturity. × 400.
Fig. 5. Beginning separation of the basal cell. × 600.
Fig. 6. Microtome section of a spore. × 1900.
Figs. 7–9. Type material of *Epicoccum nigrum*. Fig. 7. Sporodochium. × 600. Fig. 8. A young reticulate spore with the conical basal cell. × 600. Fig. 9. An irregularly shaped spore in which the pluricellularity and the conical basal cell can be seen. × 1000.
Fig. 10. *a*, *Cooke* 1842 with small sporodochia; *b*, *Cooke* 2040a with large and small sporodochia; *c*, *Cooke* 2040 with large sporodochia. All nat. size.
Fig. 11. Young sporodochium and spores of *Epicoccum andropogonis*. × 200.

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STREAM SPORA IN GHANA

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(With 1 Text-figure)

An illustrated account is given of spores found in the scum and foam of streams in three localities in Ghana. Four known species and one previously described spore type are recorded. In addition, five hitherto undescribed branched hyphomycete spores are figured.

Records have been made of the spora of a stream in Nigeria (Ingold, 1956) and also of three streams in Uganda and two in Rhodesia (Ingold, 1958*a*). A considerable difference was apparent between the species in the two regions: many species present in Britain were found in East Africa but very few in West Africa. An opportunity to enlarge the data on West African aquatic hyphomycetes occurred during a visit of Prof. C. T. Ingold to Ghana and this work was carried out with his co-operation.

Mixed foam and scum samples were taken from two streams at the foot of a scarp covered with secondary forest near Mampong, and from three streams in the Yinahin Forest Reserve, both localities being about 40 miles from Kumasi, 200 miles north of Accra. Two collections were made from a stream on the Aburi Scarp covered with secondary forest about 20 miles from Accra.

Table 1. *Aquatic hyphomycetes from Ghana*

Species	Yinahin	Mampong	Aburi
Actinospora megalospora	×	×	.
Campylospora chaetocladia	×	×	×
Flagellospora curvula	×	×	×
Triscelophorus monosporus	×	×	.
Spore forms			
Clavariopsis type, Fig. 1, D	×	.	×
Tetraradiate, Fig. 1, A	×	×	.
Tricladium type, Fig. 1, F	×	×	.
Seven-radiate, Fig. 1, B	×	×	.
Sixteen-radiate, Fig. 1, E	×	×	.
Campylospora sp., Fig. 1, G	×	×	.

Table 1 records the fungi found and their distribution within the three localities. The two northern samples were rich, both in numbers and types of spores present, but the Aburi samples contained only three species, poorly represented. *Actinospora megalospora* Ing., *Campylospora chaetocladia* Ranzoni, *Triscelophorus monosporus* Ing. and *Flagellospora curvula* Ing. were found in abundance in both northern localities, *Actinospora* being dominant.

A multi-radiate spore with fifteen or sixteen arms radiating from a central spherical part was common (Fig. 1 E) and is similar to spores described from Nigeria (Fig. 1 in Ingold, 1956). Five new spore types were found in the Yinahin and Mampong samples. A hyaline seven-radiate spore similar in basic form to the sixteen-radiate spore was common (Fig. 1 B). The spore consists of a small spherical portion bearing six or seven long ($65-75 \times 5-7\,\mu$), slender, tapering, septate arms; this differs from a seven-radiate spore from Uganda (Fig. 1 in Ingold, 1958) in not possessing a small stalk.

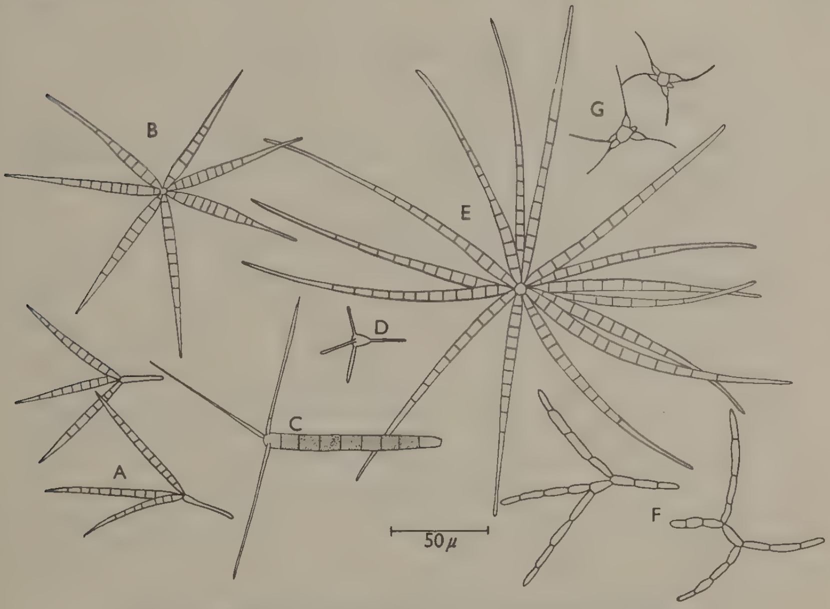

Fig. 1. Various spore-types from scum from Ghanian streams

Also well represented were four tetraradiate spores; one is similar in form to *Tricladium* (Ingold, 1942) but is composed of 15–16 cells (Fig. 1 F). The spore consists of a short arm ($25-30\,\mu$) of three cells bearing at one end of the chain a long branch ($50\,\mu$) of four cells and a single cell bearing two long arms ($35-50\,\mu$) consisting of three or four cells each. A spore type similar to a previously described form from Nigeria (Fig. 1 G in Ingold, 1956) is shown in Fig. 1 D. Similar in some respects to *Clavariopsis*, it consists of a small pear-shaped body ($8\,\mu$) bearing four long arms ($25\,\mu$), one from the narrow end and three from the swollen end. The Ghanaian spore is, however, considerably smaller than the Nigerian form. A spore type referable to the genus *Campylospora* is shown in Fig. 1 G. The spore consists of four branches similar in form and arrangement to *Campylospora chaetocladia*, but differs in being smaller with very slender ramuli. The fourth tetraradiate type (Fig. 1 A) consists of a short arm ($25-30\,\mu$) bearing three slender, tapering, septate arms ($30-40\,\mu$), the spore floating with the small arm pointing downwards.

Present in the stream spora were several slender sigmoid spores and, very commonly, a dark 8-septate spore bearing three terminal spines (Fig. 1 C), possibly a species of *Pestalotia*.

The Ghanaian collections show a fairly close similarity to those obtained in Nigeria, few British species being recorded. Several known species and spore-types were common to both countries with five new forms being observed in Ghana.

The spore types, not referable to known species, were quite different from those described from East Africa, and suggest that the aquatic hyphomycete flora of East and West Africa may be in many respects distinct.

REFERENCES

INGOLD, C. T. (1942). Aquatic hyphomycetes of decaying alder leaves. *Trans. Brit. mycol. Soc.* **25**, 339–417.

INGOLD, C. T. (1956). Stream spora in Nigeria. *Trans. Brit. mycol Soc.* **39**, 108–110.

INGOLD, C. T. (1958). Aquatic hyphomycetes from Uganda and Rhodesia. *Trans. Brit. mycol. Soc.* **41**, 109–114.

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THE DIURNAL AND SEASONAL PERIODICITY OF SPORES OF CERTAIN PLANT PATHOGENS IN THE AIR

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(With 1 Text-figure)

Using an automatic volumetric spore trap for sampling air at 2 m. above ground in an open field at Rothamsted Experimental Station, Harpenden, the diurnal and seasonal periodicities of 15 spore types belonging to fungi pathogenic to plants were followed during June to September, 1953. Large numbers of smut spores and conidia of powdery mildews occurred very early in the season when spores of *Botrytis* and *Periconia* also reached their peak. Uredospores of rusts and *Helminthosporium* spores were in maximum numbers during the middle of the season and spores of *Polythrincium* and *Epicoccum* occurred in large numbers towards the end of the season. *Alternaria* and *Stemphylium* spores began to appear during the middle of the season and persisted till the end. Spores of *Tilletia*, probably liberated into the air during the threshing operations, were seen in appreciable numbers towards the end of the season. Spores of *Ganoderma* were present over the whole period, while those of *Coniophora* occurred over a very short time. It was found that in dry weather many showed typical diurnal periodicity. *Ustilago*, *Helminthosporium*, uredospores, *Botrytis*, *Alternaria*, *Stemphylium* and *Periconia* were most abundant in the afternoon and least in the early morning. *Erysiphe*, *Polythrincium* and *Epicoccum* also occurred during the daytime but reached their maxima before noon. Spores of *Ganoderma*, *Coniophora* and other coloured basidiospores were found to occur in great concentrations between midnight and dawn. The changes observed during this crop season were compared with the existing data and it was concluded that the changes described were, in general, representative of large rural inland agricultural areas in temperate regions.

INTRODUCTION

Wind-tunnel studies of Gregory (1951) and Gregory & Stedman (1953) have shown that the gravity slide and the Petri dish exposure methods employed to study the changes in the spore content of the air by many plant pathologists and allergists are of very low efficiency. The introduction of the suction air sampling equipment, especially the Automatic Volumetric Spore Trap (Hirst, 1952), has made it possible to collect more detailed and accurate information on the changes in concentration of the air-borne spores in relation to weather, the time of the day and the season at any locality. Hirst (1953), Gregory & Hirst (1957) and Gregory & Sreeramulu (1958) have already gathered information on the diurnal and the seasonal periodicities of some common constituents of the air spora in the south of England; in this paper the diurnal and seasonal periodicities of some more air-borne spore types, the periodicities of which are less known, are described.

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METHODS

The results were obtained by operating continuously a Hirst trap at a height of 2 m. above ground in an arable field at Rothamsted Experimental Station, Harpenden, during the crop season (June–September) in 1953. Preparation of slides for exposure in the trap and mounting them for study were done according to the methods described by Hirst (1953). The slides were scanned unstained at distances along the trace corresponding to 2-hourly intervals under an oil-immersion objective. The number counted on a traverse of 40μ width was then converted into an estimated number per $m.^3$ of air. Air was sampled at a rate of 10 l./min. and the slides were changed daily at about 09.00 hr. G.M.T. The counts have been made at 2-hourly intervals starting from 10.00 hr. G.M.T. each day.

The mean diurnal periodicity curves (Fig. 1) were derived from the arithmetic means at each even hour and were plotted according to the method followed by Hirst (1953).

RESULTS

Of the fifteen spore types selected for study, the seasonal periodicities of some described by Gregory & Hirst (1957) are treated briefly, while the descriptions for others are given in detail in the following account.

Smut. Spore clumps of smuts were one of the first which appeared in large numbers at the beginning of the season. From 08.00 hr. high concentrations began to appear, reached their maximum by about noon and then by midnight decreased to a minimum and occurred in very low numbers till the next morning. During their peak season about $1000/m.^3$ were noticed at noon. High concentrations were observed only for about 3 weeks from 16 June to 6 July. A few spores of smut first appeared on 8 June and in about a week's time these higher concentrations were attained. The occurrence of smut spores in very low numbers before the first week of June and in the later part of July, August and September agrees well with the results of Sreeramulu (1956) obtained by operating a Hirst trap continuously in the centre of a smut-infected barley crop.

Powdery mildew. Conidia of *Erysiphe* were found almost all through the season. They occurred in very low concentrations during the night and from 08.00 hr. they began to appear in great numbers till noon. Their concentration gradually fell to a minimum in the evening. About $300/m.^3$ were noticed, at noon, when they were occurring in large numbers. They appeared from 5 June and higher numbers occurred only from 25 June till 15 July. They were a conspicuous element of the air spora till the middle of August and disappeared completely by the end of August.

Botrytis *type. Botrytis* is a very unspecialized common imperfect fungus occurring as a parasite on a large range of living hosts and also as a saprophyte on many dead plant materials. On the trace they were found occurring often as characteristically shrivelled, faint coloured spore clumps with much variation in the number of spores in a clump. Minimum

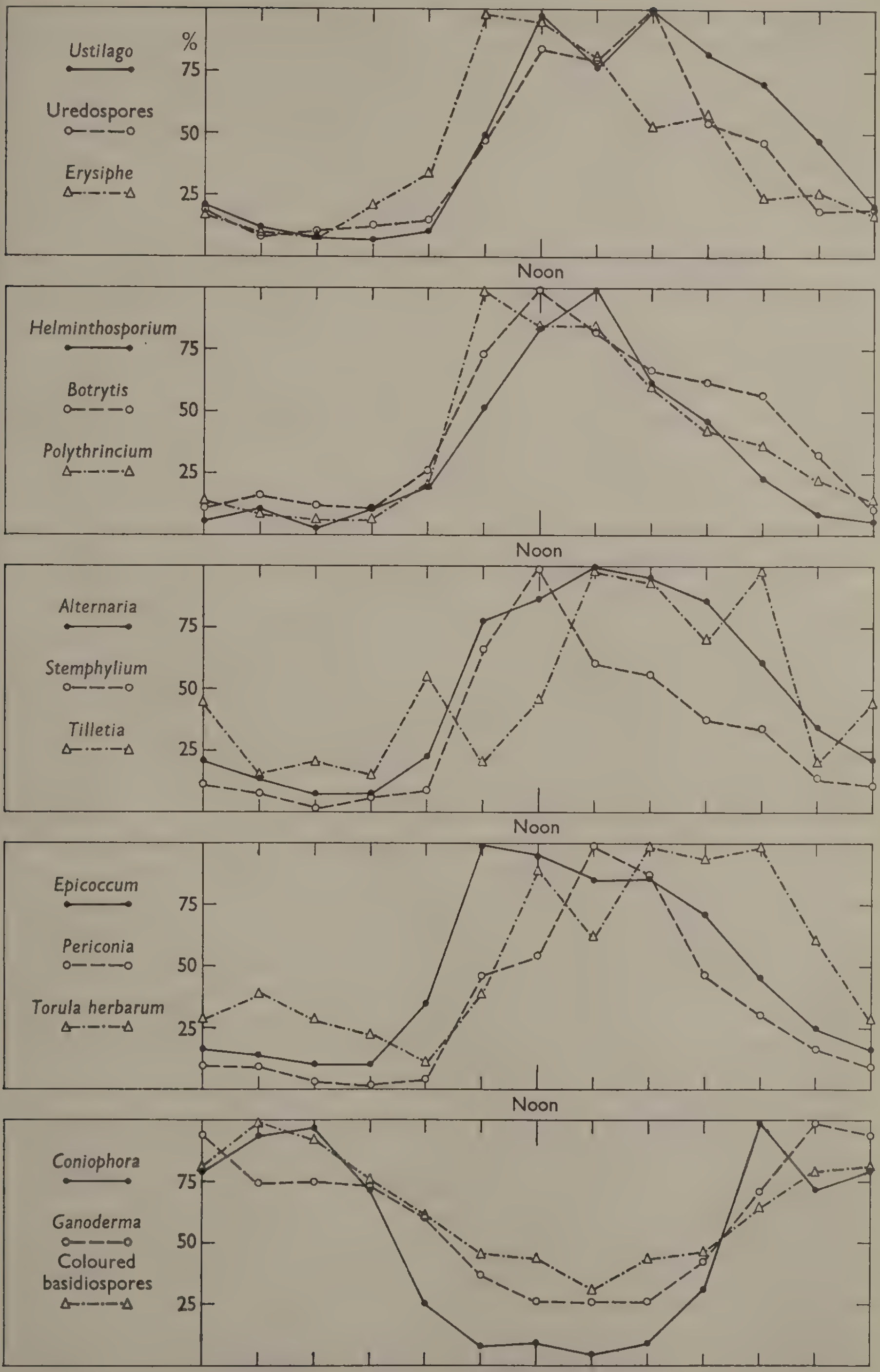

Fig. 1. Mean diurnal periodicity curves for fifteen spore types.

concentrations of these were found between midnight and dawn and a gradual increase to a daily maximum at noon (average: 400/m.^{3}) was observed. They occurred in fair numbers during the afternoon. The seasonal periodicity observed was different from many others. Spores of this type began to appear at the beginning of June, even earlier than smut spores, and maximum numbers occurred from 22 June to 2 July. During the rest of the period they occurred in low numbers.

Periconia *type*. Typical globose, one-celled, rough walled, brown conidia, probably belonging to the genus *Periconia*, a common saprophyte growing on a variety of substrata, were found in appreciable numbers even from the beginning of the season. They occurred in very low concentrations till about 10.00 hr., reached a maximum by 14.00 hr., showed a rapid fall during the afternoon, and occurred in very low concentrations from 20.00 to 08.00 hr. In their seasonal incidence they occurred in appreciable numbers even from the beginning of June and reached their peak by the beginning of July. From the end of July and in August they showed a gradual decrease in their concentration.

Uredospores. During the last 30 years the epidemiology of cereal rusts has been extensively studied in almost all the cereal growing countries of the world. The results of these studies show that in many countries the early primary infection and the later secondary spread in the crop is mainly due to the uredospores blown by wind currents. In connexion with the epidemiology of black stem rust, Craigie (1940, 1945) has made a long-term study of uredospores in the air at a number of stations in Western Canada. Although occasionally these spores were caught as early as the first week of June, usually they were not found regularly until July. In most years only in late July and August were considerable numbers found in the air. Although no active discharge mechanism is known for uredospores, Hirst (1953) found that these spores showed well-defined maximum concentrations recurring in the afternoon. The results obtained during this season confirm Hirst's observations in that maximum concentrations were found from 12.00 to 16.00 hr. and minimum during the early hours of the morning till 08.00 hr. As to their seasonal periodicity, a few spores were observed during June and July but they appeared in higher concentrations only from the beginning of August till the middle of September, when spore trapping was stopped. Considerably higher numbers were recorded during the middle of August, especially when the weather conditions were very dry.

Helminthosporium *type*. The more or less cylindrical or obclavate, multiseptate, large, brown conidia are characteristic and can be easily identified on the trace. Information concerning the presence in the air of spores of *Helminthosporium* is very meagre. For some years from 1931 data were collected on the number of spores of *H. sativum* present in the air over Manitoba in Canada by Machacek & Greaney (1938) in connexion with the occurrence of 'Kernel smudge' of cereal seeds. Except in 1935, when this disease caused much loss, they found comparatively few spores of this fungus. However, in 1935 a very marked increase in the number of these spores, especially during the last week of July and the first week of August, was noticed. Recently Grainger (1954) in Scotland has studied spore production of *H. avenae*, responsible for the leaf spot disease on oats. In his studies, extended over a period of 4 years (1946–50), he found that these spores occurred in the air only during the period of the primary and secondary phases of the disease, but none in the intervening healthy period (beginning of May until after mid-July), nor, indeed, at any other period of the year. The results obtained during the 1953 season have shown the existence of a distinct diurnal periodicity with a maximum concentration in the afternoon at Rothamsted. Higher numbers began to appear from 08.00 hr., and by 14.00 hr. they had reached their maximum; then by 20.00 hr. their concentration fell to a minimum and all through the night they were practically absent. The studies of Dillon Weston (1936) on the sporulation of *H. avenae* in artificial cultures clearly show that visible white light of a high intensity acts as a stimulus for spore production. Cultures exposed to natural light out of doors for 2 hr. 35 min. sporulated about 18 hr. later while control cultures with blackened surfaces did not sporulate. The periodicity described above suggests that light influences spore production under field conditions also. In their seasonal periodicity during this season these spores were found to be almost absent till 26 June and to occur in low concentrations throughout July. Highest numbers were found during August and they disappeared after the first week of September.

Polythrincium *type*. The dark-coloured, obovate, bicellular characteristic conidia probably belonging to *P. trifolii* which causes the 'black blotch' or 'sooty blotch' disease of clover were found in great profusion. In the U.S.A. Wolf (1935) found these conidia occurring in the air all the year round and suggested that at least in some localities these may be responsible for the primary infections also. Hirst (1953) found these conidia exhibiting a regular periodicity starting at 08.00 and reaching a peak by 10.00 hr. and suggested that this regularity may be due to the existence of a definite discharge mechanism or the daily recurrence of atmospheric conditions favouring their liberation during this time. During the 1953 season also the same type of diurnal periodicity with maxima at 10.00 hr. was observed and they began to appear from the last week of June and occurred in low numbers till mid-August. Maximum concentrations were observed from mid-August till mid-September.

Epicoccum *type*. Dark-coloured characteristic spores belonging to the genus *Epicoccum*, which is known to occur on leaves and stems of many plants, were found especially towards the end of the season in fair numbers. Spores of this type showed a periodicity with a maximum at 10.00 hr. and a minimum during the early morning. They were found in very low concentrations for a long time during the season and showed a gradual increase till the end of August when they reached their maximum. In September also they occurred in appreciable numbers.

Alternaria *type*. Spores of *Alternaria*, in addition to their ability to cause many important plant diseases, are also known to be responsible for causing respiratory dysfunction in man. The extensive surveys using the culture plate and the gravity slide methods conducted by allergists showed that in the U.S.A. these spores occur with great frequency in the air, more abundantly in certain regions than in others and that they exhibit a marked annual periodicity. The studies of Hyde & Williams (1946) showed that in Britain they occurred at all times in the year much less abundantly than other suspected allergens. They found that during 1942 and 1943 deposition of these spores at Cardiff was virtually confined to the months June to September and that catches during this period varied very greatly from day to day. In the present study *Alternaria* spores were counted under two heads—'Beaked' and 'Unbeaked' spores—to see whether the feathering effect of the elongated beak of the spore, which is believed to effect the rate of fall to a certain extent, brings about any changes in their periodicities. The diurnal periodicity curves for these two were similar except that the peak for 'Unbeaked' was reached 2 hr. later than that of the 'Beaked'. The curve for the total of the two agreed well with Hirst's curve showing the maximum concentrations during the afternoon. About 700/m.^{3} were observed during their peak hours when they were occurring in large numbers and most of them were 'Beaked' spores. Spores of *Alternaria* were practically absent till about the third week of June and from then they occurred in low numbers till the end of July. High numbers were recorded during the whole of August and in September they showed a gradual falling off. A similar seasonal periodicity for *Alternaria* was recorded by Machacek & Greaney (1938) in Canada. Durham (1938) remarked that the varying incidence of *Alternaria* spores in the U.S.A., both in time and space, depended on the availability of the straw of wheat and other cereals which seem to form the substrate for the growth of the fungus. Hyde & Williams (1946) also stated that the date of completion of the wheat harvest coincided closely with the almost complete disappearance of these spores from the air in each of the two years of their study.

Stemphylium *type*. All those spores which showed tapering at the upper end were counted as *Alternaria* even if they were not found in chains. Spores rounded at both ends, most of them having a constriction at the median transverse septum, were counted as *Stemphylium* spores following Wiltshire (1933, 1938) and Neergaard (1945). At Rothamsted during 1953 spores of *Stemphylium* type were practically absent during the night and showed a sharp peak at noon, and they occurred in low concentrations during the afternoon. In their seasonal periodicity they resembled *Alternaria* in that they were practically absent till about the end of June and occurred in large numbers from then until September. During this period whenever there was a continuous spell of dry warm weather, as on the first 2 weeks of August and the second week of September, they occurred in very high numbers.

Torula herbarum *type*. *Torula* is a saprophytic genus in which portions of, or the whole, mycelium becomes transformed into chains of spherical, dark-coloured conidia. At certain times only, considerable numbers of these conidia were found in the air during 1953. The diurnal periodicity observed for the chains of conidia indicate that they are liberated during the late afternoon, especially from 12.00 to 20.00 hr. They occurred in lowest numbers in the morning. They were never found in high numbers and were present in the air only on certain days. During this period they were found in appreciable numbers continuously only towards the end of August and the first half of September.

Tilletia *type.* Since early times *Tilletia* was recognized as one of the most important seed-borne pathogens. The studies of Heald & George (1918) indicated that at harvest time considerable numbers of these spores can be liberated into the air during threshing when the bunted grains are broken. During 15 August to 4 September 1916 they found a gradual increase in numbers present in the air, and following this maximum they recorded a gradual decline through the last week of September till they disappeared completely by October. During 1953 season at Rothamsted spores of *Tilletia* type were recorded, with daily maxima in the afternoon, from 1 August and they were found in considerable numbers during the first 2 weeks of September.

Coniophora *type.* Yellow spores of a Hymenomycete, probably *Coniophora puteana*, a common fungus causing considerable damage to structural timber, were found as a separate and quite distinctly recognizable group, in appreciable numbers. The regularity with which these spores occurred when compared with other coloured basidiospores at certain times of the day indicates a distinct night spore liberating habit in *Coniophora*. Spores of this type were found only from 19 July. They soon reached their maximum during the first week of August and occurred in high numbers till the end of August, while other basidiospores were found all through the season in varying numbers probably depending on the weather conditions. The differences observed between the *Coniophora* type and the rest clearly show the possibility of the existence of a regular spore producing phase extending over a period of about 3–5 weeks in this important wood-rotting fungus.

Ganoderma *type.* Counts were made separately of the characteristic *Ganoderma* spores (with their inner walls ornamented with thickenings in the form of a network which gives the appearance of spines extending into the outer wall) caught during this season. Only a few of these spores were observed on most days with maximum concentrations occurring at about midnight and the minimum during the daytime. A clear sharp spore-producing period as seen in *Coniophora* was not found in *Ganoderma* and these spores were seen occurring during the whole season. This may be due to the fact that spore discharge in *Ganoderma* continues over a much longer period. White (1919) recorded that in *G. applanatum* spore discharge goes on continuously day and night from May to October.

Discussion

The seasonal periodicities described above for the fifteen spore types during the 1953 crop season showed that spores of many plant pathogens occurred in appreciable numbers during the first half of the season especially when the crop plants were actively growing, while spores of saprophytic fungi such as *Alternaria*, *Stemphylium*, *Epicoccum* and *Torula* began to appear only from the middle of the season and reached their maxima when the crop plants were mature. It was also seen that a considerable number of *Tilletia* spores were found in the air at harvest time. These features are in general agreement with those recorded for the previous season by Gregory & Hirst (1957) and afford further evidence in support of the view that local sources affect the air spora in any locality and that the spore content of the air in any area mainly consists of spore types formed on dead or living vegetable matter in the locality. Also a comparison of the seasonal changes observed at Rothamsted over the two consecutive crop seasons conclusively proves that the seasonal changes observed during these two seasons, which agreed with each other, are representative of large rural inland agricultural areas in temperate regions devoted to mixed farming.

I wish to express my deep sense of gratitude to Prof. P. H. Gregory for suggesting this investigation, for his guidance and help in the preparation of this manuscript. My thanks are also due to Dr J. M. Hirst of the Rothamsted Experimental Station, Harpenden, for placing the slides exposed in the trap during the 1953 season at my disposal.

REFERENCES

Craigie, J. H. (1940). Aerial dissemination of plant pathogens. *Proc. Sixth Pacific Sci. Congr.* 1939, **4**, 753–767.

Craigie, J. H. (1945). Epidemiology of stem rust in Western Canada. *Sci. Agric.* **25**, 285–401.

Dillon Weston, W. A. R. (1936). The sporulation of *Helminthosporium avenae* and *Alternaria solani* in artificial culture. *Trans. Brit. mycol. Soc.* **20**, 112–115.

Durham, O. C. (1938). Incidence of air-borne fungus spores. II. *Hormodendrum, Alternaria* and rust spores. *J. Allergy,* **10**, 40–47.

Grainger, J. (1954). Spore production by *Helminthosporium avenae. Trans. Brit. mycol. Soc.* **37**, 412–419.

Gregory, P. H. (1951). Deposition of air-borne *Lycopodium* spores on cylinders. *Ann. appl. Biol.* **38**, 357–376.

Gregory, P. H. & Hirst, J. M. (1957). The summer air-spora at Rothamsted in 1952. *J. gen. Microbiol.* **17**, 135–152.

Gregory, P. H. & Sreeramulu, T. (1958). Air spora of an estuary. *Trans. Brit. mycol. Soc.* **41**, 145–156.

Gregory, P. H. & Stedman, O. J. (1953). Deposition of air-borne *Lycopodium* spores on plane surfaces. *Ann. appl. Biol.* **40**, 651–674.

Heald, F. D. & George, D. C. (1918). The wind dissemination of spores of bunt or stinking smut of wheat. *Bull. Sta. Coll. Wash. Agric. Exp. Sta.* no. 151.

Hirst, J. M. (1952). An automatic volumetric spore trap. *Ann. appl. Biol.* **39**, 257–265.

Hirst, J. M. (1953). Changes in atmospheric spore content: diurnal periodicity and the effects of weather. *Trans. Brit. mycol. Soc.* **36**, 375–393.

Hyde, H. A. & Williams, D. A. (1946). A daily census of *Alternaria* spores caught from the atmosphere at Cardiff in 1942 and 1943. *Trans. Brit. mycol. Soc.* **29**, 78–85.

Machacek, J. E. & Greaney, F. J. (1938). The 'black-point' or 'kernel smudge' disease of cereals. *Canad. J. Res.* **16**, 84–113.

Neergaard, P. (1945). *Danish species of* Alternaria *and* Stemphylium. Copenhagen.

Sreeramulu, T. (1956). Studies on the dispersal of spores in the air, with special reference to some pathogenic fungi. Ph.D. Thesis, University of London.

White, J. H. (1919). On the biology of *Fomes applanatus. Trans. Roy. Canad. Inst. Toronto,* pp. 159–167.

Wiltshire, S. P. (1933). The foundation species of *Alternaria* and *Macrosporium. Trans. Brit. mycol. Soc.* **18**, 135–160.

Wiltshire, S. P. (1938). The original and modern conceptions of *Stemphylium. Trans. Brit. mycol. Soc.* **21**, 211–239.

Wolf, F. A. (1935). Morphology of *Polythrincium*, causing sooty blotch of clover. *Mycologia,* **27**, 58–73.

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FUNGAL PARASITES OF THE PHYTOPLANKTON

IV. *RHIZOPHIDIUM CONTRACTOPHILUM* SP. NOV.*

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(With 4 Text-figures)

The life-history of *Rhizophidium contractophilum* sp.nov., which parasitizes *Eudorina elegans* Ehrenb., in the plankton of Windermere, and *Eudorina* sp. in Virginia Water, Surrey, is described.

The new species of *Rhizophidium* described in this paper was found on *Eudorina elegans* Ehrenb., in Windermere north and south basins during September and October 1957. It was also found by Dr B. Plunkett in the autumn of 1956 on *Eudorina* sp. in Virginia Water, Surrey. Dr B. Plunkett kindly sent me drawings he had made of the fungus to incorporate in this paper (Fig. 4), but the main description relates to the Windermere material alone.

Rhizophidium contractophilum sp.nov. (Figs. 1–4)

Thallus monocentricus eucarpicus, consistens e sporangio e zoospora encystata evoluto, e parte stirpidea non inflata intra involucrum mucosum coloniae algalis, e rhizoideo e bifurcatione unica aut e ramis brevibus paucis intra cellulam hospitis composito. Sporangium sphericum 4–10 μ diam., ad subovatum $6 \times 4{\cdot}5$–$9 \times 6\,\mu$, 4–60 zoosporas continens. Zoospora 2 μ diam., globulum parvum nitidum in parte media laterali, necnon protoplasma minus densum in parte centrali necnon flagellum posterius 10 μ long. habens; per aperturas unam vel plures (plerumque 1–3) post deliquescentiam membranae apicalis, subapicalis lateralisve perfecte formata emergens. Membrana mox post dehiscentiam collapsa atque maxima ex parte evanescens. Spora quiescens sexualiter formata velut sporangium producta, ovata $6{\cdot}5 \times 4$–$11{\cdot}5 \times 7\,\mu$, membrana crassa levi sine colore, intus subflava oleacea, e globulis pluribus parvis magnitudine aequis plurimum peripheralibus atque spatiis centralibus liquidioribus uno vel duobus praedita. Rhizoideum velut in sporangio. Thallus masculus circa 2 μ diam., ad thallum femineum per tubum conjugationis angustum plerumque agnatum aut ad 9 μ long. connexus.

Parasiticus in *Eudorina elegante* Ehrenb., in plankto lacus dicti Windemere (loci typi) et in *Eudorina* sp., loco Surrey comitatus, Virginia Water dicto.

Thallus monocentric, eucarpic consisting of a sporangium developed from the encysted zoospore, an unswollen stalk-like region within the mucilage envelope of the algal colony and a rhizoid composed of a single bifurcation or a few short branches within the host cell. *Sporangium* spherical 4–10 μ diam., or slightly oval $6 \times 4{\cdot}5$–$9 \times 6\,\mu$ containing from 4–60 zoospores. *Zoospore* 2 μ diam. with a small bright globule in the median lateral position and less dense protoplasm in the central region, flagellum posterior 10 μ long; escaping fully formed through one or more openings (usually 1–3) after deliquescence of the wall, apical, subapical or lateral.

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* Studies on British Chytrids IV.

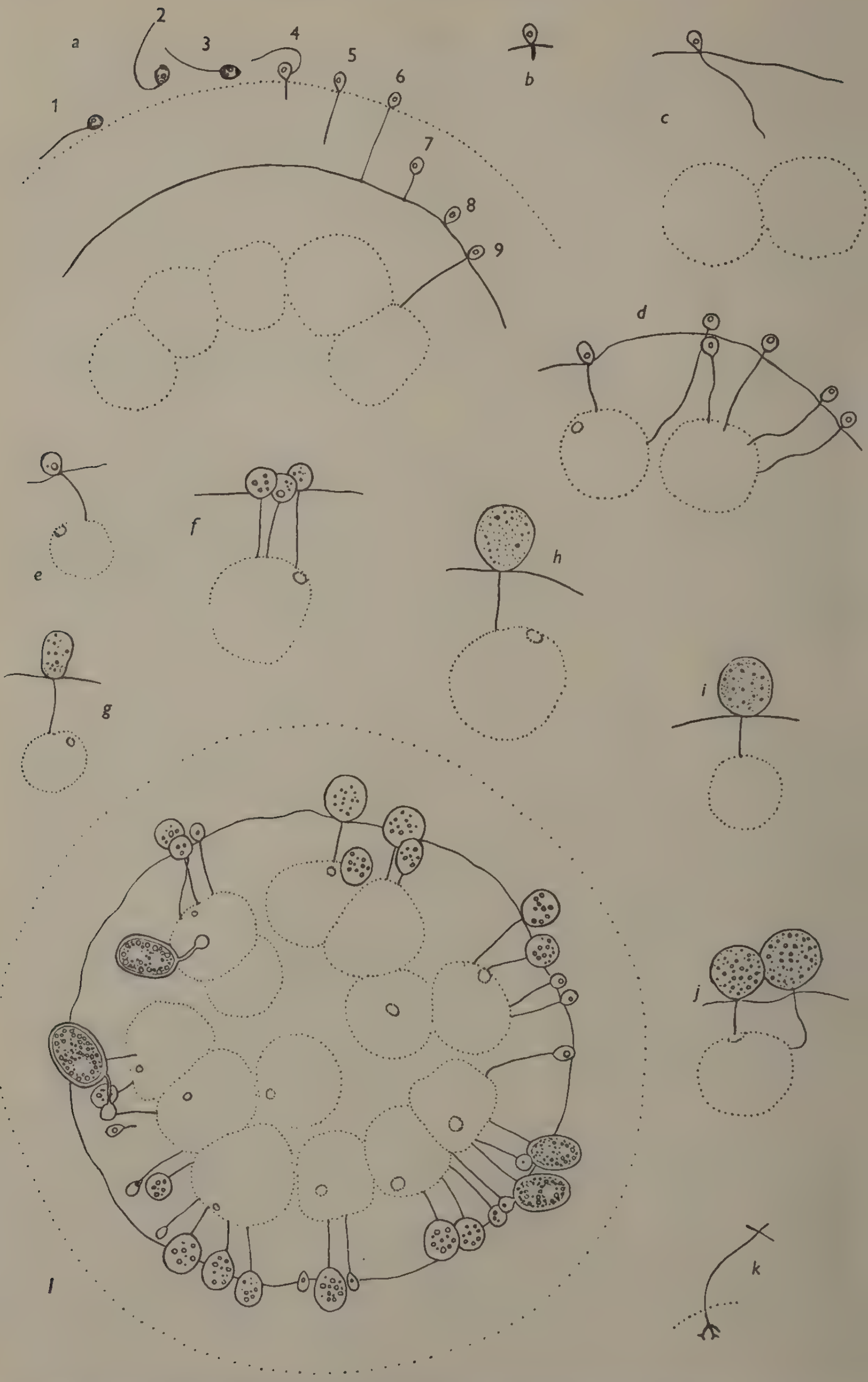

Fig. 1. *Rhizophidium contractophilum* sp.nov. *a*, stages in the passage of the zoospore to the colony membrane; *b–d*, growth of thread from zoospore to algal cell; *e–j*, stages in growth of the sporangium; *k*, branched rhizoidal system; *l*, colony bearing many infections; *k*, × 1400; rest, × 866.

Wall collapses soon after dehiscence and for the most part disappears. *Resting spore* sexually formed borne as sporangium; oval 6·5 × 4–11·5 × 7 μ, wall thick smooth colourless, content yellowish and oily composed of many small globules of equal size mostly peripheral with one or two clearer central areas. Rhizoid as for sporangium. Male thallus *c.* 2 μ diam., connected to female by a narrow conjugation tube practically adnate or up to 9 μ long.

Parasitic on *Eudorina elegans* Ehrenb., in the plankton of Windermere (type locality) and *Eudorina* sp., Virginia Water, Surrey.

The sporangium develops by enlargement of the zoospore, it is sessile on the colony membrane and embedded in the diffluent zone of mucilage. A thin thread-like structure extending through the colony mucilage connects the rhizoidal system inside the algal cell with the sporangium. In order to reach the colony membrane the zoospore has to travel a distance of up to 16 μ which is accomplished as follows: the zoospore becomes quiescent on the outer edge of the diffluent mucilage with its flagellum parallel to the contour of the mucilage. The flagellum may still vibrate (Fig. 1 *a*, 4). After a few minutes the contents of the cell look abnormal as if it were dying. Then gradually there appears a fine thread extending from the zoospore body towards the colony membrane (Fig. 1 *a*, 5). While this thread elongates the flagellum may still move. By the time the thread has reached the surface of the colony it is easily visible. The flagellum by now has disappeared and slowly the zoospore body is drawn towards the interior of the host colony by contraction of the thread (Fig. 1 *a*, 7). The closer it comes to the colonial membrane the faster its movement seems to be. This journey through the mucilage takes about 30 min., ending when the zoospore reaches the surface of the inner, more densely mucilaginous membrane of the colony (Fig. 1 *a*, 8). It is now oval rather than spherical but still contains its single globule. No further development may take place for several hours, after which a second thread is produced which makes contact with the host cell (Fig. 1 *a*, 9). It must take many hours for this germ thread to dissolve the colony membrane and grow to the host cell, for although I have watched many zoospores traverse the outer mucilage I have never been able to watch long enough to see one develop a germ thread. Once contact is made with a *Eudorina* cell the zoospore begins to enlarge into a sporangium. During development small refractive globules accumulate in the protoplasm (Fig. 1 *f*), later they disappear and the content becomes uniformly greyish and granular. Following this, small bright granules appear in the previously non-refractive protoplasm (Fig. 1 *i*). These enlarge and eventually form the oil globules of the zoospores which are randomly distributed in a clear matrix. The rhizoidal system is minute and can only be seen under the highest magnifications after decolorizing the alga. It is located immediately within the cell wall and consists of a single bifurcation or a few short branches (Fig. 1 *k*). Mature sporangia (Fig. 2 *b*–*e*) are spherical 4–10 μ diam., or oval 6 × 4·5–9 × 7 μ and contain from 4 to 60 zoospores. As dehiscence approaches the sporangium wall appears to become thinner and loses its refractiveness. The wall deliquesces in an irregular manner and the zoospores, fully formed, emerge through one or more openings, usually 1–3 (Fig. 2 *f*–*h*). Sometimes when the subapical part deliquesces

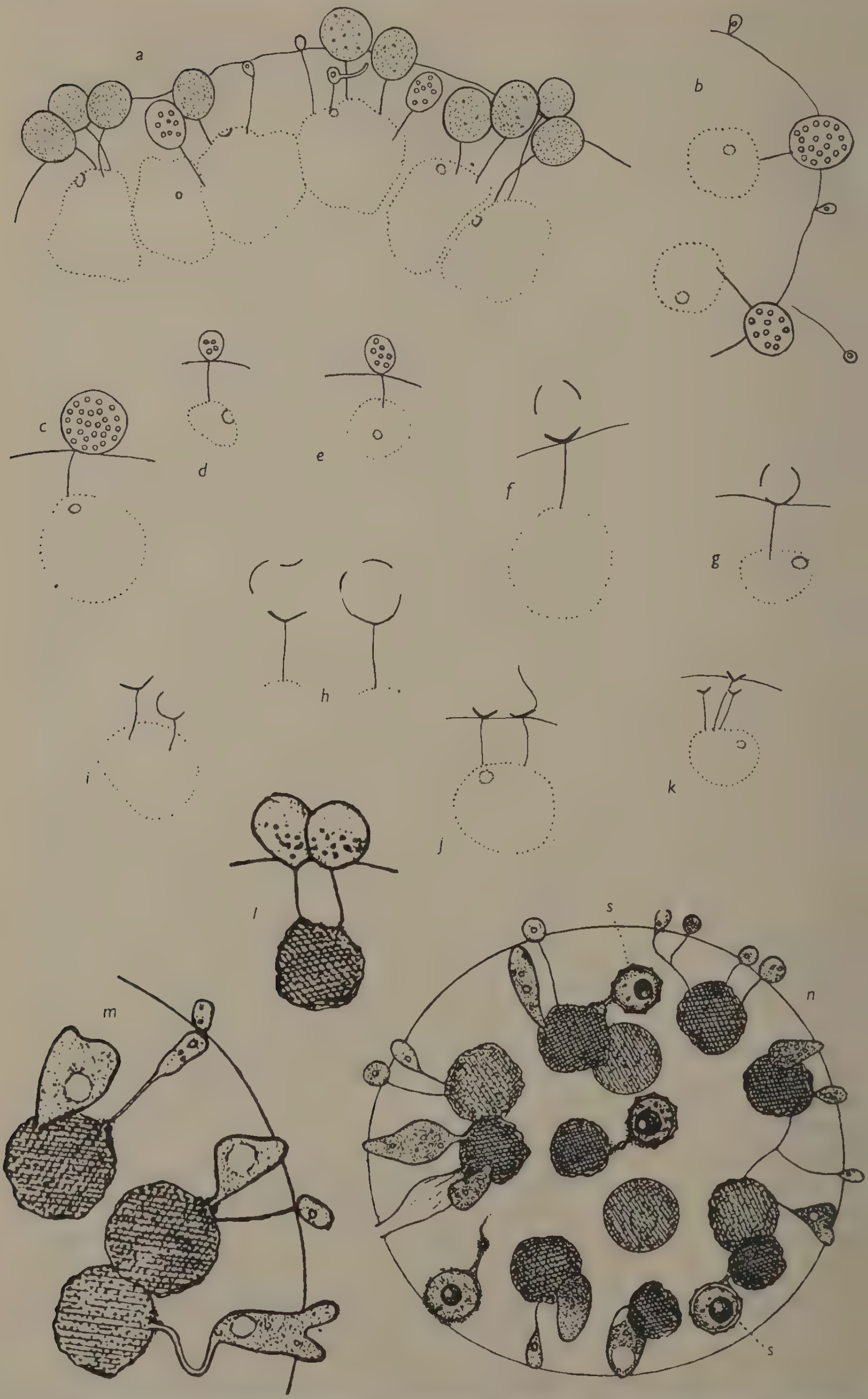

Fig. 2. *Rhizophidium contractophilum* sp.nov. *a*, Portion of a colony showing numerous sporangia at the same stage in development; *b–e*, mature sporangia; *f–h*, sporangia immediately after dehiscence; *i–k*, stages in deliquescence of the wall; *l–n*, *Rhizophidium eudorinae* Hood (after Hood, 1910). *a–k*, ×866; *l–m*, ×700; *n*, ×500.

first the entire circumference at this level must be involved, for the dome-shaped apex of the sporangium is seen to be lying freely in the medium, resembling a lid.

After it leaves the sporangium the zoospore passes through the diffluent mucilage of the host colony into the external medium. The zoospore (2 μ diam., flagellum 10 μ long) is spherical when swimming, but may be ovate when quiescent. The protoplasm is less dense in the central region than elsewhere (Fig. 1 *a*, 1–3). There is a single, small, conspicuous globule in the median lateral position. After reaching the external medium it may immediately swim away with a smooth, gliding movement and frequent changes in direction, or it may remain just outside the mucilage. In the latter case its flagellum still vibrates and the cell exhibits changes of shape and spasmodic changes of position. The sporangium collapses soon after the first few zoospores have emerged and then almost immediately the remaining parts of the wall begin to dissolve (Fig. 2 *i*, *j*). After about half an hour only the basal portion is visible (Fig. 2 *k*). Later, this too must disappear for *Eudorina* colonies bearing witness to past parasitism by the presence of empty sporangia cannot be found. Visually, the contents of cells bearing a sporangium do not differ from uninfected ones. The flagella still move and the chloroplast and eyespot appear normal. It seems therefore that a single infection is insufficient to kill the cell as it does in *Loborhiza metzneri* Hanson and *Rhizophidium oblongum* Canter. This may be because the absorbing organ occupies a comparatively small area in the host cell. However, on dehiscence, many zoospores remain close to the empty sporangium and germinate very soon after their liberation. Thus a colony with only a few primary infections may soon become covered with sporangia and also resting spores (Fig. 1 *l*), and then the whole or part of the colony dies. The fungus does not produce any striking colour changes in the algal chloroplast. The colour first changes from bright to pale green and finally the whole cell becomes more or less colourless. Bacteria then flourish in the mucilage (Fig. 3 *s*). Resting spores develop early in the period of fungal infection. They are sexually formed and conjugation is anisogamous. The male resembles an encysted zoospore which has produced a germ thread to the host cell. A conjugation tube grows along the surface of the colony to the female gamete (Fig. 3 *a-d*) which resembles a very young sporangium. Sometimes the male cell is more or less adnate to the female, hence a conjugation tube is virtually absent (Fig. 3 *f*, *h*). Occasionally two males make contact with one female. The content passes from the male into the female; sometimes a granule in Brownian movement is left. The young resting spore contains a few globules but as it grows the number increases; in shape it becomes more elongate than the sporangium; the content becomes more oily and seems to contract from the outer wall leaving a clear peripheral region (Fig. 3 *h*, *i*). Later an inner wall forms around the margin of the contracted content (Fig. 3 *j*, *q*). Now the resting spore looks yellowish; this is not due to coloration of the smooth wall but to the oily content. The latter consists of many small globules of equal size, mostly peripheral, with one or two central clearer areas (Fig. 3 *q*). One resting spore with a large central globule was seen (Fig. 3 *k*) and whether this represents the mature condition is unknown. The resting spore is

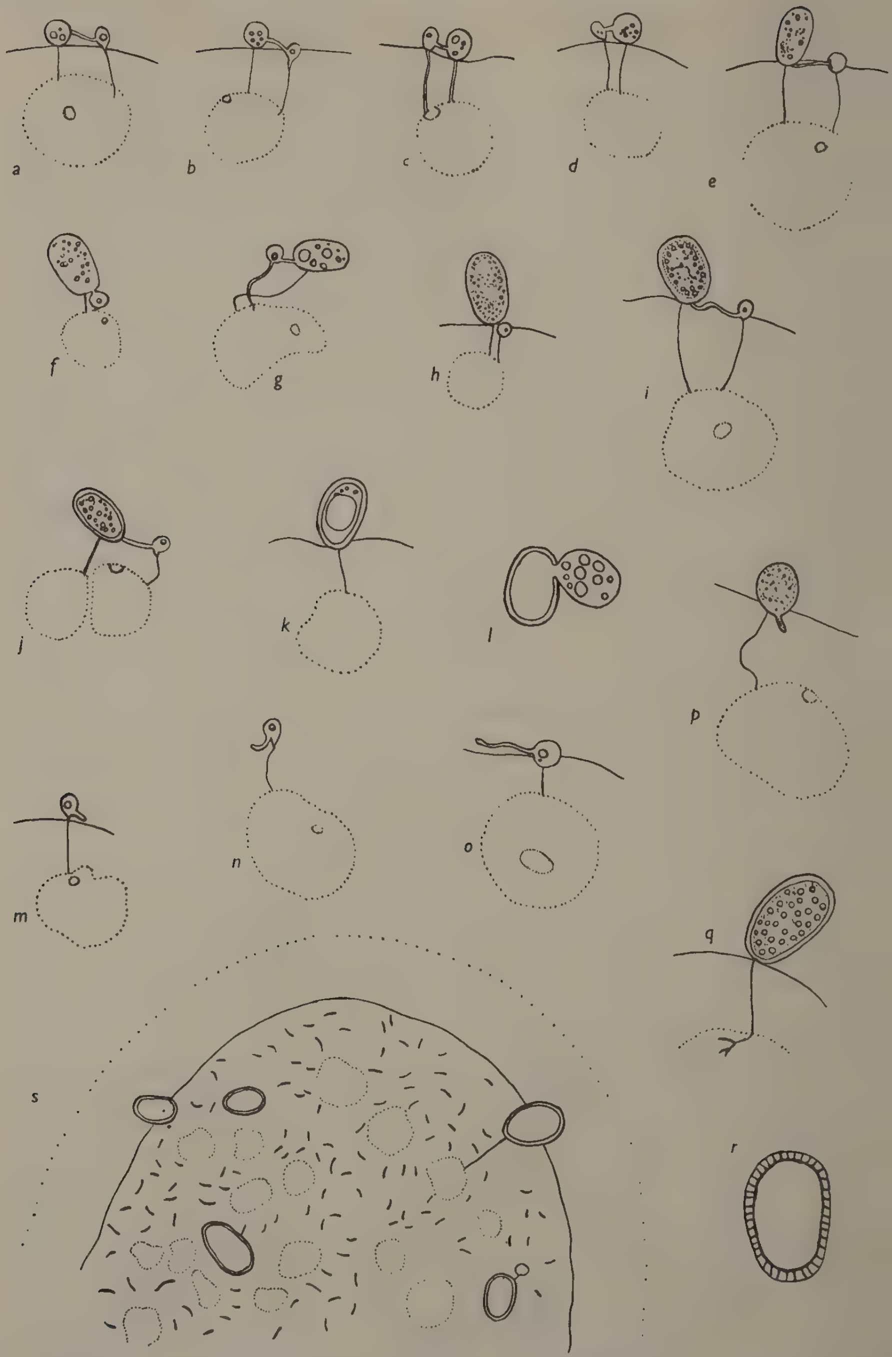

Fig. 3. *Rhizophidium contractophilum* sp.nov. *a–j*, Stages in development of the resting spore; *k*, ? mature specimen with single globule; *l*, ? germinating resting spore; *m*, *n*, male thalli which will probably grow into sporangia; *o*, *p*, male thalli definitely growing into sporangia; *q*, resting spore in glacial acetic acid to show rhizoid; *r*, ? parasitized resting spore; thicker inner wall with rod like processes, content omitted; *s*, disintegrating colony with germinated resting spores and bacteria. *q* and *r*, × 1400; rest × 866.

elongate oval in shape and varies in size from 6·5 × 4 μ to 11·5 × 7 μ. In some resting spores the inner wall developed rod-like processes, and became thicker and more refractive than the outer wall (Fig. 3*r*). It is possible that these resting spores contained resting spores of *Rozella* sp. This idea did not occur to me at the time of study so no confirmation is possible.

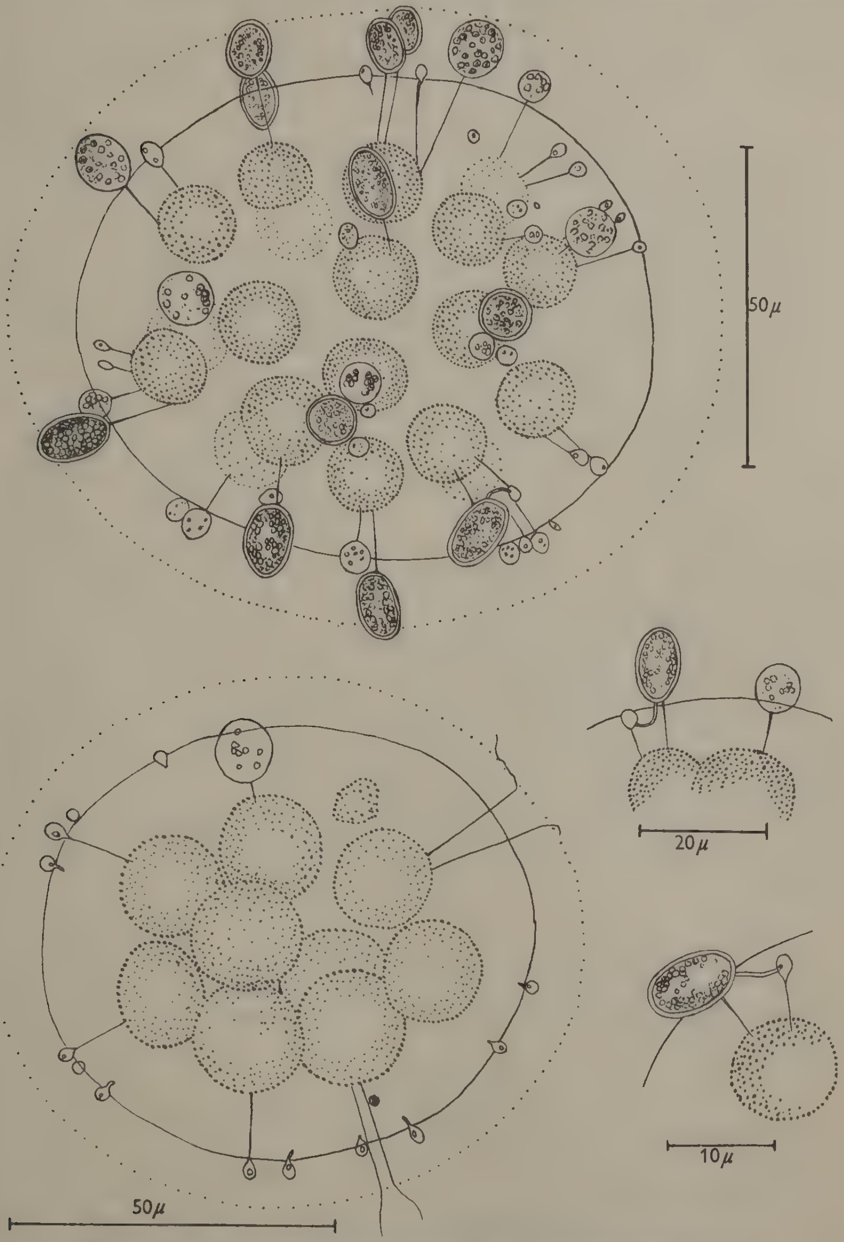

Fig. 4. *Rhizophidium contractophilum* sp.nov. from Virginia Water, Surrey. Drawn by Dr B. Plunkett.

However, it does seem likely since the resting spores pictured from Virginia Water (Fig. 4) all had smooth walls.

The rhizoidal system of the resting spore is similar to that of the sporangium (Fig. 3*q*). Whether the germ thread of the male has any branched threads inside the host cell is unknown. *Eudorina* colonies were found bearing empty, smooth-walled, resting spores (Fig. 3*s*) which presumably had germinated. Many resting spores had a greyish granular content in small dense pieces, rather than oily and yellowish. These may represent a stage in the breakdown of the oily content prior to germination. Only one germinating resting spore was found (Fig. 3*l*) and it behaved as a prosporangium. It is not unusual for a male which has not made contact with a female or been arrested in its development to proceed to develop as a sporangium (Fig. 3*o*, *p*).

Although many chytrids have already been described on *Eudorina* and its near relative *Pandorina*, the species here described does not conform to any of them. It does, however, bear a striking similarity to two sporangia pictured by Hood (1910, fig. 5): see Fig. 2*l*. She described them as abnormalities of *Rhizophidium eudorinae* Hood. I quote p. 5: 'Certain abnormalities were observed in the form of the parasitic cells some of which showed indications of longitudinal division at the apex, a wide apical cleft making its appearance. In other cases the cell had completely divided in a transverse direction.' It seems more probable that the latter represents two separate infections of *R. contractophilum* or a similar chytrid. In a previous discussion on Hood's paper (Canter, 1946, p. 133) I suggested that *R. eudorinae* may represent material of *Dangeardia mammillata* Schröder together with an epibiotic chytrid, i.e. ?*R. contractophilum*. Even so the spherical resting spores Hood described (see Fig. 2*n* (*s*)) cannot be accounted for. It is possible that the main description (i.e. the flask-shaped sporangia and spherical rough-walled resting spores) represents a fungus similar in development to *Loborhiza metzneri* Hanson or *Phlyctidium eudorinae* Gimesi. Then the abnormalities described as cleft specimens (Fig. 2*m*) could be interpreted as early stages in the formation of the resting spore by a similar sexual process to that found in the above two species. At the moment this possibility has only a theoretical basis but there is no doubt that as more material of *Eudorina* is examined from different localities the mysteries of *R. eudorinae* will be solved.

I am most grateful to Dr J. W. G. Lund for help with the manuscript and to Dr Hanna Croasdale for the Latin translation. I also acknowledge that the apparatus used in this investigation was obtained from the Central Research Fund of the University of London.

REFERENCES

Canter, H. M. (1946). Studies on British Chytrids. I. *Dangeardia mammillata* Schröder. *Trans. Brit. mycol. Soc.* **29**, 128–134.

Hood, O. (1910). On *Rhizophidium eudorinae*, a new chytridiaceous fungus. *Proc. Bgham. nat. Hist. Soc.* **12**, 38–45.

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THE NUTRITION OF *PHYTOPHTHORA FRAGARIAE*

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(With 1 Text-figure)

An investigation was made into the nutritional requirements of three physiologic races of *Phytophthora fragariae* using homogenized mycelium as inoculum.

The optimal calcium concentrations for growth on a complex amino-acid medium were between 20 and 50 p.p.m. No growth occurred in the absence of Ca ions. Strontium could partially replace Ca for two of the isolates but Ba was wholly ineffective. The Ca requirements of the fungi were independent of the nature of the C or N sources used. Glucose, maltose, sucrose and dextrin were good C sources for the fungi. Fructose, mannose and raffinose were utilized to a limited extent. Organic acids and amino acids were not used as C sources by *P. fragariae* 11. The fungi did not use nitrate or ammonium N, even in the presence of organic acids and over a fairly wide range of initial pH values. Of 18 amino acids tested, singly, for ability to support growth, DL-alanine, L-proline, L-asparagine, DL-serine, DL-valine and DL-aspartic acid proved to be good sources of N. Ammonium ions inhibited the growth of *P. fragariae* 11 in a medium containing DL-alanine as the N source.

Earlier work on *Phytophthora fragariae* Hickman revealed a number of interesting nutritional requirements. Hickman (1940) found that it was restricted in its ability to use common laboratory culture media, and although growth occurred on oat and French bean agars, it was very slow in comparison with six other *Phytophthora* species. Later, Fleetwood Walker (1955), working with one isolate of *P. fragariae*, showed it to be highly exacting in its N requirements, the only favourable N sources being casein hydrolysate or a complex amino-acid mixture of approximately the same composition. In addition, growth on the latter N source was shown to be dependent on the addition of Ca ions at the optimal rate of 70 p.p.m. Since few fungi were known to require Ca and organic N, the nutrition of *P. fragariae* was considered worthy of a further study, part of which is reported here.

MATERIALS AND METHODS

The three isolates of *P. fragariae* differed in their pathogenicity to the strawberry cultivars, Huxley, Perle de Prague and Climax. Isolate 11 attacked only Huxley, isolate 29 attacked both Huxley and Perle de Prague while isolate 10 was pathogenic to all three cultivars.

Media

The basal medium (medium B) was a modification of that used by Fleetwood Walker (1954) and had the following composition: Glucose 5·0 g., KH_2PO_4 0·5 g., $MgSO_4.7H_2O$ 0·25 g., thiamin 1·0 mg., Fe

0·2 mg., Zn 0·16 mg., Cu 0·04 mg., Mn 0·02 mg., Mo 0·014 mg., distilled water to 1000 ml. (The micro-nutrients were added as 1 ml./l. of a trace element stock solution.)

Medium HC was prepared by the addition of 2·5 g. of vitamin-free casein hydrolysate to a litre of medium B.

Medium SA, a complex amino-acid medium used by Fleetwood Walker and based on published analyses of casein hydrolysate, had the following composition: Glycine 60 mg., L-lysine 180 mg., DL-serine 140 mg., L-cystine 8 mg., DL-valine 160 mg., DL-leucine 200 mg., L-proline 250 mg., DL-alanine 70 mg., DL-phenylalanine 110 mg., DL-methionine 60 mg., L-glutamic acid 500 mg., DL-aspartic acid 160 mg., DL-threonine 110 mg., DL-tyrosine 140 mg., L-histidine 70 mg., L-arginine 90 mg., DL-*iso*-leucine 140 mg., medium B to 1000 ml.

The symbol Ca 40 affixed to a medium designation indicates the presence of Ca ions (added as chloride) at the rate of 40 mg./l. The carbohydrate source and the Ca salt were autoclaved separately and added to the bulk of the medium when cold. Unless otherwise stated, all media were adjusted to an initial pH of 6·7 with NaOH and HCl.

Preparation and level of inoculum

Small discs cut from the edge of an actively growing colony on an agar medium were used by Fleetwood Walker as inoculum. This technique was known to suffer from a number of disadvantages such as high coefficients of variation between replicates and also an erratic lag phase, sometimes of 10 days, between inoculation and the start of active growth. Zoospores proved to be an impracticable inoculum since the three isolates showed marked differences in their ability to sporulate under identical conditions and also the sporulating capacity of each isolate was found to vary erratically over a period of time. Mycelial homogenates proved to be a suitable inoculum, and after preliminary experiments the following procedure was adopted.*

Mycelial mats from five 14-day-old cultures grown in 20 ml. of medium HC were suspended in 60 ml. of sterile distilled water and blended for 10 sec. at 6000 r.p.m. in the sterilized, monel-metal container of an M.S.E. 'Atomix' blender. The resulting homogenate was washed three times by centrifugation and finally resuspended in 80 ml. of sterile distilled water. The amount of mycelium per ml. was determined turbidimetrically with reference to a previously prepared calibration curve, and the volume of the suspension adjusted to bring the mycelium concentration to 1 mg. dry weight/ml. Inoculations were made at the rate of 1 ml. of this suspension per flask.

Incubation and measurement of growth

The fungi were grown in 20 ml. of liquid medium in 100 ml. 'Pyrex' conical flasks capped with 1¼ in. diam. aluminium 'Oxoid' caps. All cultures were incubated at 20° C. Static culture was used throughout since all three isolates reacted unfavourably to the conditions of submerged

* I have found this technique to be successful with other species of *Phytophthora* and *Pythium*.

shaken culture. Dry-weight determinations were generally made after 14 or 21 days incubation. The dry weights presented in the tables and figures represent the average of duplicates.

RESULTS

The effect of pH *and alkaline earth metals on growth*

The effect of adjusting the initial pH of medium SA. Ca40 on the growth of the fungi is seen in Fig. 1. All three isolates exhibited a sharply defined growth optimum at pH 6·8, and their growth was severely limited below pH 5·8 and above pH 7·2.

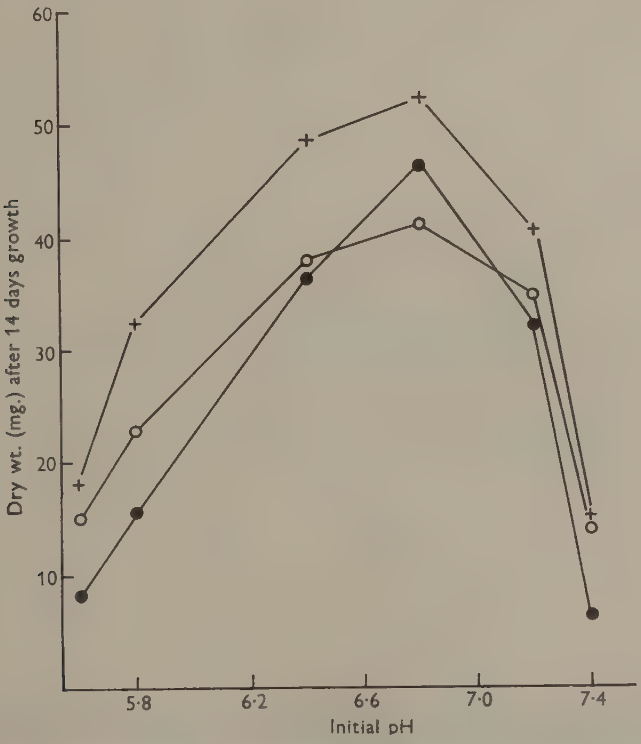

Fig. 1. Influence of the initial pH of medium SA. Ca40 on the growth of *P. fragariae*. ○—○, isolate 10; +—+, isolate 11; ●—●, isolate 29.

The growth of the three isolates was compared on medium HC and medium SA with and without the addition of Ca ions. The results presented in Table 1 confirm those obtained by Fleetwood Walker and reveal that a Ca requirement was demonstrable only when a synthetic amino-acid mixture was used as the N source. Flame photometric determinations showed, however, that Ca ions were present in medium HC at the rate of 20 mg./l. The removal of Ca from casein hydrolysate was effected by precipitation with a calculated quantity of 0·1 M sodium oxalate. Growth of the fungi on medium HC prepared from Ca-free casein hydrolysate was found to be dependent on the addition of Ca ions at 20–40 mg/l. Later experiments, using a wide range of media, showed that the Ca requirements of the fungi were independent of the nature of the C or N sources used.

The relationship between growth and Ca concentration in medium SA is shown in Table 2. For all isolates, 20 and 50 p.p.m. were the most favourable Ca concentrations, although the response of *P. fragariae* 11 was greater than that of the other isolates. No growth occurred in the absence of Ca and concentrations of the metal ion in excess of 50 p.p.m. were markedly inhibitory.

Table 1. *The effect of calcium ions on the growth of* Phytophthora fragariae *in media HC and SA*

Medium	Dry wt./flask (to nearest mg.) after 14 days incubation		
	Isolate 10	Isolate 11	Isolate 29
HC	48	54	45
HC+Ca^{++} (40 p.p.m.)	41	48	41
SA	±	±	±
SA+Ca^{++} (40 p.p.m.)	48	58	50

± = Trace of growth; *c.* 2–3 mg. dry wt.

Table 2. *The effect of the calcium concentration on the growth of* Phytophthora fragariae *in medium SA*

Calcium concentration (p.p.m.)	Dry wt./flask (to nearest mg.) after 14 days incubation		
	Isolate 10	Isolate 11	Isolate 29
0	±	±	±
5	35	34	32
10	39	38	41
20	39	61	45
50	43	64	50
100	21	23	25
200	18	18	16

± = Trace of growth; *c.* 2–3 mg. dry wt.

In a number of instances it has been found that the Ca requirements of a micro-organism can be partially satisfied by Sr and Ba (Basu, 1951; Ingraham & Emerson, 1954). Table 3 shows that Sr added to medium SA at levels of 20 and 50 p.p.m., permitted suboptimal growth of isolates 11 and 29 but was without effect on the growth of isolate 10. Ba could not replace Ca for any of the isolates, but since the addition of Ba to the medium resulted in the precipitation of barium sulphate the validity of this conclusion is doubtful.

Carbon requirements

Fleetwood Walker (1954) tested glucose, fructose, sucrose, maltose, dextrin and starch as C sources for *P. fragariae* 11. Starch was the only one not utilized. In the present investigation, the compounds listed in Table 4 were tested as C sources, the concentration of each being adjusted to give a C level equivalent to that of medium SA. Ca 40 containing 5 g. of glucose/l. Of the monosaccharides, only glucose was utilized to any appreciable extent. The disaccharides, maltose and sucrose, and the polysaccharide, dextrin, proved to be good sources of C. Organic acids and amino acids were not used by *P. fragariae* 11.

Table 3. *The effect of calcium, strontium and barium ions on the growth of* Phytophthora fragariae *in medium SA*

	Dry wt./flask (to nearest mg.) after 14 days incubation		
Supplement	Isolate 10	Isolate 11	Isolate 29
Ca, 20 p.p.m.	41	59	45
Ca, 50 p.p.m.	48	62	49
Ca, 100 p.p.m.	25	27	24
Sr, 20 p.p.m.	±	29	13
Sr, 50 p.p.m.	±	12	6
Sr, 100 p.p.m.	±	±	±
Ba, 20 p.p.m.	0	0	0
Ba, 50 p.p.m.	0	0	0
Ba, 100 p.p.m.	0	0	0

± = Trace of growth; *c.* 2–3 mg. dry wt.

Table 4. *Growth of* Phytophthora fragariae *in medium SA. Ca40 containing carbon sources other than glucose*

	Dry wt./flask (to nearest mg.) after 14 days incubation		
Carbon source	Isolate 10	Isolate 11	Isolate 29
Glucose (control)	40	51	42
Glycerol	0	±	0
Xylose	4	5	6
Fructose	9	11	13
Galactose	±	9	7
Mannose	12	17	15
Sorbose	0	0	0
Maltose	37	46	35
Lactose	±	5	±
Sucrose	42	49	46
Raffinose	16	25	20
Dextrin	28	38	32
Starch	7	11	8
Sodium acetate	—	±	—
Sodium succinate	—	±	—
Sodium citrate	—	0	—
DL-Alanine	—	±	—
L-Asparagine	—	±	—
L-Glutamic acid	—	±	—

± = Trace of growth; *c.* 2–3 mg. dry wt.

Nitrogen requirements

Ammonium nitrate, ammonium chloride and potassium nitrate were tested as N sources in medium B. Ca40 at levels of 0·28 and 0·056 % N. Portions of each medium were adjusted to initial pH values of 4·2, 5·5 and 6·5. None of these media supported the development of the isolates, although traces of growth were observed in media containing ammonium nitrate. The addition of tartaric, malic, citric and succinic acids, singly, to portions of medium B. Ca 40 containing ammonium nitrate as the N source had no effect on the capacity of the medium to support growth of the fungi. Thus, the inability of *P. fragariae* to use either nitrates or ammonium salts, even in the presence of organic acids and over a fairly wide range of pH values, was confirmed. However, from Table 5 it will be seen that a considerable amount of growth did occur in medium B. Ca 40 supplemented with certain single amino acids at the rate of 3 g./l.

Table 5. *Growth of* Phytophthora fragariae *in medium B. Ca* 40 *supplemented with single amino acids*

	Dry wt./flask (to nearest mg.) after 21 days incubation		
Supplement	Isolate 10	Isolate 11	Isolate 29
L-Asparagine	39	37	41
DL-Aspartic acid	36	24	34
L-Glutamic acid	20	22	20
Glycine	±	5	4
DL-Alanine	43	48	46
DL-Valine	29	34	31
DL-Leucine	24	24	26
DL-*iso*-Leucine	17	19	14
DL-Serine	32	34	36
DL-Threonine	10	9	8
DL-Phenylalanine	4	6	6
DL-Tyrosine	0	0	0
L-Proline	36	39	39
L-Histidine*	22	22	24
L-Arginine*	9	12	12
L-Lysine*	±	6	±
L-Cystine	0	0	0
DL-Methionine	13	12	11
Medium HC (control)	51	65	55

* Added as mono-hydrochloride.
± = Trace of growth; *c.* 2–3 mg. dry wt.

The amino-acid requirements of the isolates were remarkably similar, the only obvious difference being the greater utilization of aspartic acid by isolates 10 and 29. There appeared to be no correlation between the structure of an amino acid and its utilization by the fungi. For example, the amino acids generally supporting good growth included a straight chain amino acid (alanine), a branched chain compound (valine), a cyclic amino acid (proline), a β-hydroxy amino acid (serine), an acid amide (asparagine) and a dicarboxylic amino acid (aspartic acid).

The effect of ammonium ions on the growth of P. fragariae 11

French (1953) and Saksena & Bhargava (1943) found that ammonium ions had an inhibitory effect on the growth of *Phytophthora infestans* and *P. phaseoli*, respectively. The data presented in Table 6 show that this is also true for *P. fragariae* 11. Similar results were obtained when ammonium ions were supplied in the form of ammonium sulphate. The inhibition was most marked when DL-alanine was used as the N source. The reason for the alleviating effect of succinate on ammonium inhibition is unknown but, in view of the pH data presented, the phenomenon can hardly be explained in terms of the extra buffering capacity of the medium

Table 6. *The effect of ammonium ions on the growth of* Phytophthora fragariae 11

(The organic nitrogen sources and supplements were added to medium B. Ca 40 at the following concentrations: L-glutamic acid 0·01 M, DL-alanine 0·02 M, NH_4Cl 0·04M, and Na-succinate 0·2 %, w/v. The initial pH of each medium fell within 6·6–6·8.)

Organic N source	Supplement	Final pH	Dry wt./flask (to nearest mg.) after 21 days
—	NH_4Cl	6·35	0
—	NH_4Cl + Na-succinate	6·25	0
L-Glutamic acid	—	7·1	23
L-Glutamic acid	NH_4Cl	6·25	18
DL-Alanine	—	5·5	61
DL-Alanine	NH_4Cl	6·3	14
DL-Alanine	NH_4Cl Na-succinate	6·1	26

Discussion

Of the *Phytophthora* species tested for their ability to grow on various sources of N (Wills, 1954; Lopatecki & Newton, 1956), only three have been reported to require organic N. One, *P. infestans*, has been shown by French (1953) to grow on a medium containing asparagine, while another, *P. phaseoli*, requires alanine for growth (Saksena & Bhargava, 1943). Finally, Fleetwood Walker (1955) demonstrated a complex organic N requirement in *P. fragariae.* In the present investigation, although the N requirement was shown to be less complex than was originally thought, the inability of the fungus to use inorganic N was confirmed. Cantino (1955) has suggested that ammonium salts might well have served as N sources for both *P. infestans* and *P. phaseoli* if rigid pH control had been maintained by adequate buffering. While it is true that the reaction of the medium can influence the utilization of inorganic N (Morton & Macmillan, 1954), in view of the experiments reported here this proviso can hardly be applied to *P. fragariae.*

Relatively few micro-organisms have been shown to have an absolute requirement for Ca ions (Steinberg, 1948). Definite Ca requirements have been reported for species of *Allomyces* and *Saprolegnia* (Machlis, 1953; Ingraham & Emerson, 1954; Reischer, 1951), *Trichophyton interdigitale* (Mosher, Saunders, Kingery & Williams, 1936) and *Phytophthora fragariae* (Fleetwood Walker, 1955). The present investigation has shown that the latter fungus requires Ca ions under all the conditions tested and in quantities characteristic of macro- rather than micro-nutrients. Although an increasing number of fungi have been reported to require Ca for growth, little is as yet known about its role in fungal metabolism. The results of a more detailed investigation into the effects of Ca ions on the growth and respiration of *P. fragariae* 11 will be published later.

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REFERENCES

Basu, S. N. (1951). Significance of calcium in the fruiting of *Chaetomium* species, particularly *Chaetomium globosum*. *J. gen. Microbiol.* **5**, 231–238.

Cantino, E. C. (1955). Physiology and phylogeny in the water molds—a reevaluation. *Quart. Rev. Biol.* **30**, 138–149.

Fleetwood Walker, P. M. (1954). Physiology of Phycomycetes with particular reference to *Phytophthora fragariae* Hickman. Ph.D. thesis, University of Birmingham.

Fleetwood Walker, P. M. (1955). Observations on the physiology of *Phytophthora fragariae*. Abstr. in *Trans. Brit. mycol. Soc.* **38**, 169.

French, A. M. (1953). Physiologic differences between two physiologic races of *Phytophthora infestans*. *Phytopathology*, **43**, 513–516.

Hickman, C. J. (1940). The Red Core root disease of strawberry caused by *Phytophthora fragariae*, n.sp. *J. Pomol.* **18**, 89–118.

Ingraham, J. L. & Emerson, R. (1954). Studies on the nutrition and metabolism of the aquatic Phycomycete, *Allomyces*. *Amer. J. Bot.* **41**, 146–152.

Lopatecki, L. E. & Newton, W. (1956). The nutrition of *Phytophthora*. *Canad. J. Bot.* **34**, 751–757.

Machlis, L. (1953). Growth and nutrition of water molds in the sub-genus *Euallomyces*. I. Growth factor requirements. *Amer. J. Bot.* **40**, 189–195.

Morton, A. G. & Macmillan, A. (1954). The assimilation of nitrogen from ammonium salts and nitrate by fungi. *J. exp. Bot.* **5**, 232–252.

Mosher, W. A., Saunders, D. H., Kingery, L. B. & Williams, R. J. (1936). Nutritional requirements of the mould *Trichophyton interdigitale*. *Plant Physiol.* **11**, 795–806.

Reischer, H. S. (1951). Growth of Saprolegniaceae in synthetic media. I. Inorganic nutrition. *Mycologia*, **43**, 142–155.

Saksena, R. K. & Bhargava, K. S. (1943). Nitrogen requirements and vitamin deficiencies of *Phytophthora phaseoli* Thaxter. *Proc. Indian Acad. Sci.* B, **18**, 45–51.

Steinberg, R. A. (1948). Essentiality of calcium in the nutrition of fungi. *Science*, **107**, 423.

Wills, W. H. (1954). The utilisation of carbon and nitrogen compounds by *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker. *J. Elisha Mitchell Soc.* **70**, 231–235.

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Trans. Brit. mycol. Soc. 42 (2), 201–212 (1959).

INFECTION OF PEAS BY *FUSARIUM SOLANI* VAR. *MARTII* FORMA 2 AND THE SPREAD OF THE PATHOGEN

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(With Plate 10 and 10 Text-figures)

Early Bird and Laxton's Superb peas were inoculated by growing them in compost into which had been stirred suspensions of *Fusarium solani* 'conidia' from submerged, shaken culture. The fungus normally infected the cotyledons, foot and roots. Sporodochia formed on the foot-rot lesion and testas. All the roots were susceptible to penetration, but the progress of infection was very slow. Aerial parts were also infected under very humid conditions. *F. solani* conidia were shown to be dispersed by air currents from dry sporodochia, on particles of dry soil to which they adhere and in drops of water splashed from sporodochia. In nonsterile compost, *F. solani* mycelium from infected roots spread only to immediately adjacent roots. On glass slides in garden soil at different water-holding capacities, the fungus did not grow but formed chlamydospores.

INTRODUCTION

Foot-rot of peas caused by *Fusarium solani* (Mart.) Sacc. var. *martii* (Appel & Wollenw.) Wollenw. f. 2 Snyder, hereafter referred to as *F. solani*, is a widely known disease. It was first recorded by Jones (1923) in the United States of America and subsequently in Britain by Ogilvie (1929), in Holland by Went (1934), in Germany by Snyder (1935), in New South Wales (1943) and in New Zealand (1949).

The term 'foot' has arbitrary limits and denotes the top of the tap root, the hypocotyl, the base of the epicotyl and, when present, the cotyledons. The foot-rot lesion is variable in extent and may be confined below soil level or extend for a short distance above it. Observations on pea foot-rot made by numerous other workers have been limited largely to the shape and colour of the conspicuous foot-rot lesion, and the factors affecting the degree of pathogenicity such as type and concentration of inoculum and the particular isolates of *F. solani* used. In the present work an attempt was made to obtain a more complete picture of the host-parasite relationship by studying the susceptibility of the whole plant to penetration by *F. solani*, the general progress of infection and the further saprophytic development of the fungus in the host. The dispersal of *F. solani* was also examined as it has so far received little attention. The only published work on this aspect of its biology is that of Jones (1923), who postulated that the mycelium persisted in and spread through the soil.

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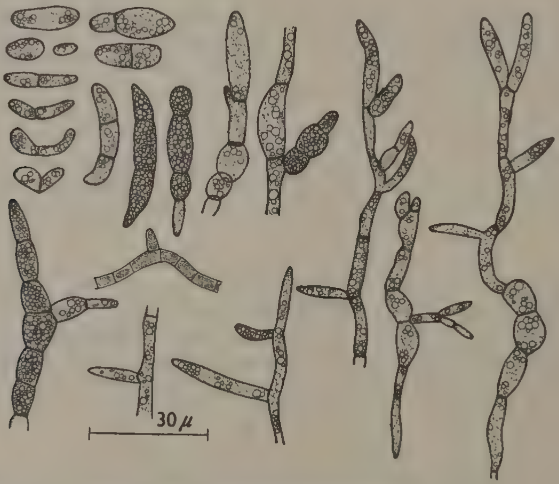
MATERIALS AND METHODS

The experiments were made with two cultivars of pea, Early Bird and Laxton's Superb, using an authentic culture of *F. solani* obtained from Prof. J. C. Walker, University of Wisconsin, U.S.A. Inocula consisted of aqueous suspensions of micro- and macroconidia, which were stirred into compost so that the conidia were distributed throughout. At first, potato-dextrose agar cultures were used as the source of conidia, but these could not always be relied upon to produce sufficient inoculum. Resort was then made to the conidia-like cells (Text-fig. 1) of *F. solani* which were produced in a liquid potato-dextrose medium* shaken in a reciprocating shaker 125 times per minute for 3 days at 20° C. Foot-rot symptoms like those found in the field were produced satisfactorily by both forms of inoculum.



Text-fig. 1. 'Conidia' and mycelial fragments of *F. solani* produced in submerged, shaken culture at 20° C.

INFECTION AND SYMPTOM DEVELOPMENT

The penetration of Early Bird peas by *F. solani* and the path of infection was followed microscopically and by means of isolations from infected plants.

Surface sterilization of the tissue was a necessary precaution when making isolations and, in order to determine in which region of the plant initial penetration occurred, a sterilant was required which would be lethal to only superficial growth of *F. solani*. Silver nitrate was chosen since it can be inactivated quickly by precipitation of AgCl with NaCl, and the minimum time of exposure was determined at which $AgNO_3$ was lethal to mycelium, macroconidia and mycelium inside slices of infected pea tissue two to three cells thick. Immersion for 20 sec. in 1·0 % $AgNO_3$

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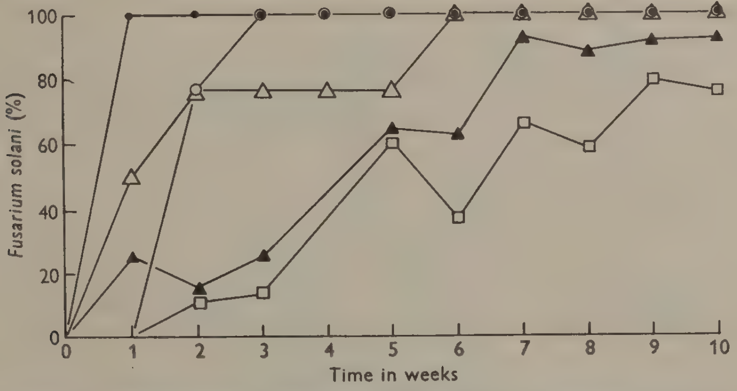
* 2 g. dextrose and water extract from 20 g. potato tissue per 100 ml.

followed by two washings in 5% NaCl and two washings in sterile distilled water was found sufficient.

Laxton's Superb peas were grown in inoculated compost in a greenhouse at 60° F. At weekly intervals for 10 weeks the root systems of four plants were drawn to scale, and twenty-four to thirty 1 cm. lengths of root and foot per plant were excised from marked positions, surface sterilized as described above, plated on potato-dextrose agar and incubated at 25° C. The positions from which *F. solani* grew were noted on the drawings. The frequency with which *F. solani* was isolated from the epicotyl, hypocotyl, root base and tap root, respectively, and the progress of infection, is shown in Text-fig. 2. After 1 week, *F. solani* had penetrated the hypocotyl, root base and one or two places along the tap root. After 2 weeks it was isolated, in addition, from the base of the epicotyl and, during the following weeks, its frequency of isolation increased progressively, first from the tap root and secondly from the lateral roots. The root systems of 9-week-old plants were almost completely invaded by *F. solani*.

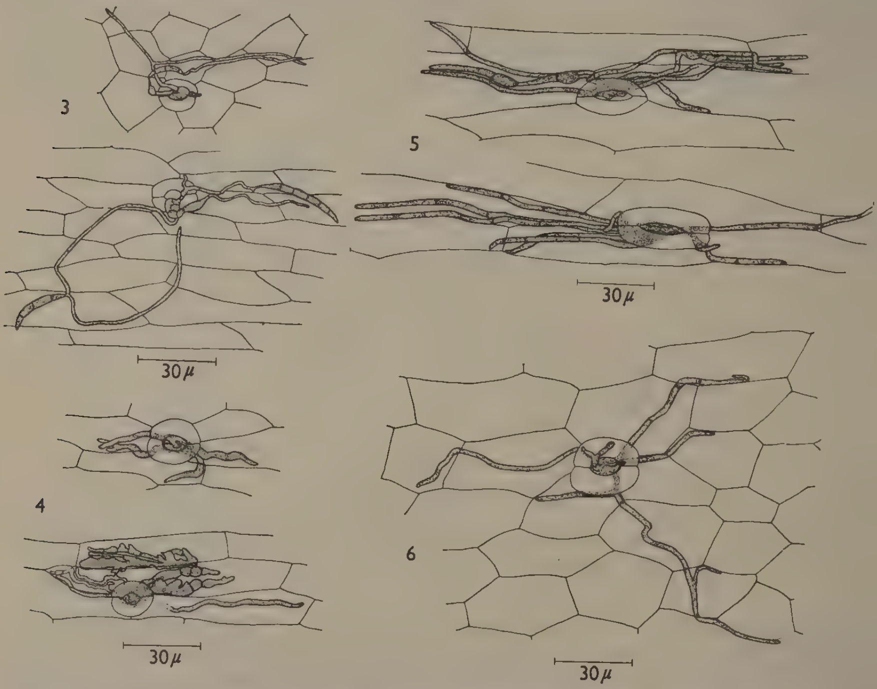


Text-fig. 2. The frequency of isolation of *F. solani* and the progress of infection. ○, Epicotyl; ●, hypocotyl; △, root base; ▲, tap root; □, lateral roots.

It was clear from these observations that the foot was infected first and most severely, whereas the roots were infected gradually, comparatively mildly and at scattered points. In addition, it was observed that damaged tissue, for example the cortex at the origin of the lateral roots, soon became discoloured dark brown to black, and *F. solani* was isolated from these lesions.

The first visible symptoms on the plants appeared after 1 week as minute, black flecks scattered thinly on the epidermis of the cotyledon stalks, on the underground part of the epicotyl, the hypocotyl and the uppermost part of the tap root. Flecking of the foot region was also noticed by Padwick (1938). Examination of thin, tangential slices of epidermis and outer cortex showed that a small, newly established mycelium was associated with each fleck and the brown to black discoloration was pronounced in the cell walls. The flecks were frequently associated with stomata. Successive stages in penetration were followed using preparations stained in acid fuchsin and mounted in lactophenol.

One or more germ tubes from a macroconidium entered a stoma and formed a small knot of hyphae under the epidermis, blocking the stomatal pore (Text-fig. 3). After the establishment of mycelium inside the host, the conidia and germ tubes became detached and hyphae grew inter- and intracellularly into the surrounding tissue (Text-fig. 4). Where the cells were elongated, as in the epicotyl base, the mycelium grew longitudinally (Text-fig. 5) and the external flecks were long. Where the cells were more or less isodiametric, as in the hypocotyl, the mycelium grew out more or less radially (Text-fig. 6) and the flecks were not elongated. Isolations from the flecks confirmed the presence of *F. solani*.

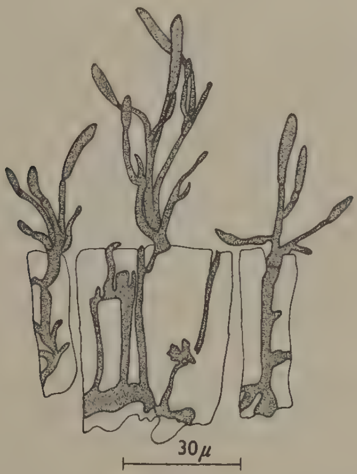


Text-figs. 3–6. Text-fig. 3. Penetration through stomata by *F. solani*. In the lower figure the knot of hyphae has been pulled out of the stoma. Text-fig. 4. Young mycelia of *F. solani* in host tissue. Text-fig. 5. Elongated mycelia of *F. solani* in the base of the epicotyl. Text-fig. 6. Mycelium of *F. solani* growing more or less radially in the hypocotyl.

After 2–3 weeks the mycelia of the flecks had grown, coalesced and formed a lesion which encircled the foot. Transverse sections showed that the mycelium was inter- and intracellular and confined to the epidermis and outer cortical cells. The marginal hyphae advanced up the epicotyl to above soil level and downwards into the top of the tap root forming the foot-rot lesion. Small sporodochia were seen on tangential slices of some of the coalesced flecks of 2-week-old plants, and larger sporodochia, which arose from mycelium in the diseased cortex and bore macroconidia, were seen on sections of the foot lesions on plants 4–5 weeks old (Pl. 10, fig. 1). The mycelium in the foot region was dense and gradually grew deeper into the cortex. After 5–6 weeks the steles were frequently discoloured brick-red and hyphae had penetrated as far as the pericycle.

Hyphae in the roots did not grow so vigorously as those in the foot and, generally, the mycelium was sparse and did not cause visible symptoms. However, where the mycelium was dense, for instance in the damaged tissue at the origin of the lateral roots, there was greyish to black discoloration.

Isolations from dark brown to black flecks on the testa and cotyledons showed that they were infected after 1 week. Stomata were not found in the epidermis of the cotyledons. After 2 weeks the lesions on the cotyledons enlarged and became more or less circular. Sporodochia arose on the outside of the testa from inter- and intracellular mycelium of the palisade, subepidermal and sclerenchyma cells. Young macroconidia on branched conidiophores arising from mycelium in the palisade layer are shown in Text-fig. 7. Under greenhouse conditions the testas disintegrated after about 3 weeks.



Text-fig. 7. *F. solani* conidia on branched conidiophores growing from palisade cells of pea testa. Preparation stained with acid fuchsin in lactophenol and mounted in lactophenol.

Although infection of the aerial parts was not normal, that it was possible for *F. solani* to penetrate the vines and leaves under very humid conditions was shown as follows. The aerial parts of Early Bird plants were sprayed with a suspension of 'conidia' and kept at 68° F. under a bell jar. After 1 week the leaves and stems were turning yellow and there were occasional dark flecks on the stems. Inter- and intracellular and superficial mycelium, which bore sporodochia with macroconidia, was abundant on the yellowed parts. Small mycelia were associated with the dark flecks on the stems. The uninoculated plants remained vigorous and were of a healthy green colour. Similar results were obtained after 8 days at 60° F. when the aerial parts were buried in inoculated and uninoculated compost.

The general growth and cropping of plants infected by a soil-borne inoculum of conidia was scarcely different from that of healthy plants although, after 6 weeks at 60° F., there was a slight reduction in vine length as shown in Pl. 10, fig. 2. These mild symptoms occurred whether the suspension contained 1 or 4000 conidia/ml. and the reason was presumably because the fungus was confined to the cortex during the growth and development of the plants until they were nearly mature. In addition, the root systems were as extensive as the controls and only mildly infected. New lateral roots were healthy at first as they arose endogenously from healthy tissue.

THE SPREAD OF *FUSARIUM SOLANI*

During the infection experiments, it was noticed that foot-rot occurred very frequently amongst the control plants and this focused attention on the question of the spread of the parasite. It was possible that *F. solani* was present in (i) imperfectly sterilized compost, or (ii) pea seed, or (iii) that it was dispersed by airborne conidia from sporodochia which were observed on artificially inoculated plants in the greenhouse and out-of-doors and also on naturally infected plants in the field. Some observations were made on each of these possibilities. Spread through the soil could not have occurred in these experiments since the inoculated and uninoculated plants were always in separate pots, but some tests were carried out to determine whether *F. solani* could grow through the soil and infect roots (iv).

(i) *Contaminated compost.* A compost mixture of 7 loam:2 sand:1 peat contained in 5 in. pots was autoclaved at 15 lb. pressure for 1 hr. Particles from the surface and centre of the autoclaved compost were placed on plates of malt-extract agar. Particles from similar positions in non-autoclaved compost were plated out in the same way. Mycelium did not grow from the autoclaved compost, whereas it grew in abundance from the non-autoclaved, thus showing that this method of sterilization was thorough.

Two pots of sterile compost planted with Early Bird peas and two with Laxton's Superb were placed in a greenhouse at 65–70° F. where infection tests were in progress. In due course all the plants growing in the previously sterilized compost developed foot-rot lesions from which *F. solani* was isolated. The origin of this infection was clearly not the compost.

(ii) *Seed-borne infection.* Thirty pea seeds from the stock used in the tests were incubated at 77° F. on plates of potato-dextrose agar. By the time the seeds had germinated and formed seedlings a variety of fungi had grown out but none was *F. solani*. The conclusion that the seeds were not infected was further borne out by the results of the test described below.

(iii) *Airborne dispersal.* The possibility of infection by airborne conidia was investigated. Early Bird and Laxton's Superb peas planted in sterile compost (autoclaved at 15 lb. pressure for 1 hr.) were grown in a greenhouse (*a*) among plants infected with *F. solani*, (*b*) about 4 ft. away from these infected plants on the opposite side of the greenhouse, and (*c*) in a second greenhouse where no experiments with *F. solani* had been carried out. The peas were grown at 65–70° F. in each situation.

After 30 days all the plants in the greenhouse containing the infected plants had foot-rot lesions, whereas those in the other greenhouse were unaffected. *F. solani* was isolated consistently from the lesions of plants grown in the same greenhouse, (*a*) and (*b*), but was never isolated from the plants grown in the second greenhouse, (*c*). If the infection had been seed-borne, foot-rot would have appeared on the plants in the second greenhouse. It was thus concluded that *F. solani* was dispersed through the air for at least 4 ft.

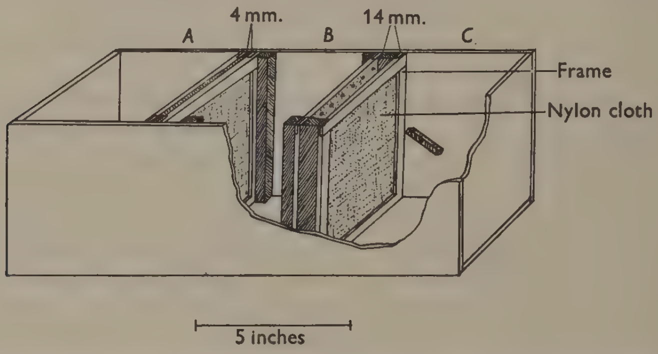
When Early Bird and Laxton's Superb peas were planted in unsterile compost and grown in the second greenhouse in the absence of infected plants, no infection by *F. solani* occurred. The unsterilized compost was, therefore, free from *F. solani*.

The possibility that conidia from sporodochia on infected pea tissue and conidia washed into soil could become airborne was now examined. Sterile air was gently blown over (i) wet sporodochia on pea tissue, (ii) dry sporodochia on pea tissue, (iii) moist, inoculated compost, (iv) dry, inoculated compost, and (v) dry, uninoculated compost, on to plates of potato-dextrose agar which were subsequently incubated at 77° F. Colonies of *F. solani* appeared on the agar plates of series (ii) and (iv) only. In series (iv) every colony grew around a soil particle. Conidia can, therefore, be dispersed by air currents from dry sporodochia and on dry soil particles to which they adhere. Having thus shown that the conidia could be airborne, it remained to demonstrate their presence in the air of the greenhouse and this was achieved using slide spore traps. When slides coated thinly on one side with lubricating oil were placed about 3 in. away from infected pea plants for 24 hr., three or four macroconidia were found per slide as compared with none on slides arranged similarly near healthy plants.

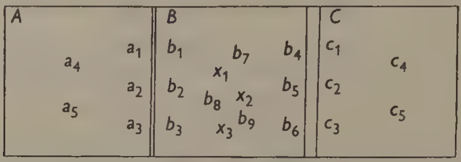
The possibility that conidia may be carried for short distances in drops of water splashed from sporodochia on infected pea tissue was examined. Ten drops of distilled water, ± 0·01 ml. per drop, were released approximately 50 cm. above sporodochia. Every splash contained conidia and it was found that, under these conditions, conidia could spread for ± 44 cm.

F. solani also spread out-of-doors from inoculated to control plants 3 ft. away. After 4 weeks occasional control plants had *F. solani* foot-rot and after 6 weeks all were infected. Macroconidia were found on the foot lesions.

(iv) *Spread through the soil.* The possibility of mycelial growth through the soil from infected to healthy roots was examined by separating the roots with a nylon mesh through which mycelium but not roots could pass. Taut sheets of nylon cloth supported on frames were arranged 4 and 14 mm. apart as shown in Text-fig. 8, and duplicate boxes were prepared. The spaces between the nylon sheets were filled with unsterilized compost. Ten 4-week-old healthy Early Bird peas (a_1–a_5 and c_1–c_5) were planted in unsterile compost in each of the compartments *A* and *C* as shown in Text-fig. 9. The roots of plants a_1–a_3 and c_1–c_3 were placed immediately against the nylon cloth, while the remaining plants (a_4, a_5, c_4 and c_5) were planted in the centre of the compartments as controls. Nine 4-week-old infected Early Bird plants were planted in unsterile compost in compartment *B*. The roots of three plants were placed immediately against the nylon cloth at each end of the compartment, plants b_1–b_3 and b_4–b_6, respectively, and the roots of three other infected plants, b_7–b_9, were intertangled with the roots of three healthy plants, x_1–x_3, and planted in the centre of compartment *B*. The compost was covered with rubberized sheeting to eliminate airborne infection and the plants were grown in the greenhouse at 60° F. for 5 weeks. After this time the soil against the nylon sheets was carefully pushed back and the roots on the exposed surface



Text-fig. 8. Diagram showing the arrangement of nylon sheets and frames inside the box.



Text-fig. 9. Plan of the distribution of pea plants in the box shown in Text-fig. 8.

were removed, cut into 1 cm. lengths, surface sterilized and plated on potato-dextrose agar. The remaining roots were then removed and 1 cm. lengths were plated out as before.

The results of the duplicated experiment are given in Table 1 and show that the initially healthy roots in contact with infected roots became severely infected; healthy roots separated from infected roots by 4 mm. were only slightly infected, *F. solani* being isolated from 19·1 and 9·5 % root pieces, respectively; healthy roots separated from infected roots by 14 mm. and over were not infected.

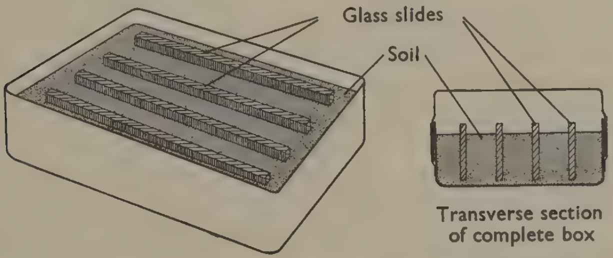
These results indicate that *F. solani* can spread slowly in unsterile soil by root contact and not by growth through the soil in the absence of the host.

Mycelial growth and also the behaviour of macroconidia were further examined in sterile and unsterile garden soil sieved through a 0·1 mm. mesh sieve and brought to different percentage water holding capacities

(W.H.C.), using a technique based on a development of the Rossi-Cholodny technique described by Conn (1932). Microscope slides were placed in plastic soap boxes as shown in Text-fig. 10. The % W.H.C. of the sieved soil was determined by the perforated box method of Keen & Raczkowski (1921). Mycelium and conidia were introduced on to the slides by placing rectangles of potato-dextrose agar 1 × 2 cm. containing the fungus on one end of slides and incubating in a humid atmosphere in a covered glass vessel for 4 days. A fringe of hyphae about 7 mm. wide grew along the slide and, together with a considerable number of conidia, remained attached to the slide when the agar was removed. The limit of the fringe was marked by ruling a line in indian ink on the back of the slide. A known constant weight of the soil was introduced into each box and four slides bearing mycelium and conidia were inserted into the soil. This was achieved without dislodging the fungus if the front of the slide was protected by holding another slide near it, while pushing it into the soil. Immediately the slides were in position water was added from a burette to produce the following range of % W.H.C.: 0, 20, 40, 60, 80 and 100. The lids were then fitted on and sealed with cellophane tape to reduce evaporation. The loss of water after 5 days at 68° F. from soil at 100 % W.H.C. reduced the W.H.C. by only 0·25 % and was negligible.

Table 1. *Isolations of* Fusarium solani *from infected roots*

Compartment	Plant	No. root pieces	No. *F. solani* colonies	% *F. solani* colonies	No. root pieces	No. *F. solani* colonies	% *F. solani* colonies
A	a_1–a_3	21	4	19·1	21	2	9·5
	a_4–a_5	24	0	0	24	0	0
B	b_1–b_9	30	26	86·5	30	18	60·0
	x_1–x_3	26	6	22·2	33	16	48·5
C	c_1–c_3	24	0	0	24	0	0
	c_4–c_5	21	0	0	21	0	0



Text-fig. 10. Views of the boxes with soil and microscope slides in position.

Two slides from each box were examined after 4 and 8 days, respectively. Each slide was removed by first pressing the back gently against the soil, which broke contact between the soil and the mycelium, and then pulling it out vertically. The box could be closed and resealed with a reduction in the W.H.C. of only 0·04 %. Mycelial growth did not occur at any W.H.C. in the unsterile series, but occasional growth up to 2 mm.

occurred in the sterile series at 20–60 % W.H.C. After 8 days nearly all the mycelium in both series had disintegrated; in the unsterile at 20–100 % W.H.C. and in the sterile at 40–100 % it was replaced by chlamydospores. Many of the macroconidia either developed chlamydospores or formed a short germ tube which terminated in a chlamydospore. *F. solani* mycelium grew along the parts of the slides projecting above the soils at 20–100 % W.H.C. The viability of the mycelium, macroconidia and chlamydospores on the slides after 8 days was tested by growth in potato-dextrose agar. Thin, rectangular pieces of this agar were pressed gently on to the fungus and left in position for 24 hr. at 68° F. The fungus could be seen through the agar and, although bacteria had multiplied, they did not obscure vision. No growth occurred from the mycelial fragments, but the macroconidia and chlamydospores germinated and started to grow into the agar.

Discussion

The conditions of infection in the experiments carried out in this work were designed to resemble as closely as possible the postulated conditions of infection in the field, in that the inoculum of conidia-like cells was distributed evenly throughout the soil in which the peas were subsequently planted so that all the underground parts of the plants were equally exposed to the fungus. Periodic isolations from the roots and foot regions of plants infected in the experiments showed that *F. solani* penetrated all the underground parts. The symptoms on the roots were very faint and the hyphae inside the host were sparse except where the tissue was damaged as, for instance, at the origins of the lateral roots where there was dark brown to black discoloration and dense hyphae. The points of penetration in the foot region, however, were marked by dark brown to black flecks which were also noticed by Padwick (1938). A further interesting observation here was that germ tubes from macroconidia frequently entered through stomata. The mycelium in the foot region was very dense and, as it grew, the areas of dark coloration enlarged and coalesced until the characteristic, irregularly shaped foot-rot lesion finally formed. The aerial parts of plants were also shown to be susceptible to infection, but only under very humid conditions.

When the mycelium was well established inside the host sporodochia developed. No previous record of sporodochia of *F. solani* on peas has been discovered, but sporodochia of *F. anguioides*, *F. arthrosporioides* and *F. sporotrichioides* were found by Togashi (1928) on the foot lesions of peas.

Since the whole plant was susceptible to infection, it was curious that a clearly defined, dark-coloured lesion developed only on the foot. There are four possible explanations of this: (1) the foot was in the soil from the time of germination and was subjected to infection for longer than the other parts; (2) it was adjacent to the cotyledons which were infected very soon after planting; (3) the number of entry points was greater in the foot region than in the roots because penetration occurred through stomata; and (4) the foot region may be a particularly favourable environment, which was indicated by the very dense growth there, as compared with the roots.

The mild symptoms caused by *F. solani* may be attributed to the relatively slow development of the fungus inside the host. The mycelium in the foot region, though dense, remained confined to the cortex until the host was nearly mature, and in the tap and lateral roots infection occurred only at scattered points and the hyphae were usually sparse. The slight reduction in the functional root surface was, in addition, partly offset by the growth of new healthy roots through diseased cortex, a feature also observed by Walker & Snyder (1933). Similar mild infection was characteristic of all the isolates of Bockman (1950) in Germany, but Buxton (1955) in England found that some isolates were more and some less pathogenic than this. However, when a very heavy mycelial inoculum was used, pre- and post-germination rot, as observed by Davies (1943), occurred.

The widespread distribution of *F. solani* foot-rot in the field points to a means of dispersal more efficient than the slow mycelial growth postulated by Jones (1923) and, in fact, the limited experiments designed to examine the ability of the fungus to grow through soil gave no support to his suggestion. No growth of mycelium was observed but, on the contrary, hyphae and macroconidia on buried slides formed chlamydospores. Possibly, in the field, an inoculum of chlamydospores may be built up in soil from mycelium once living saprophytically in pea tissue. Although there was no evidence of spread below ground, apart from that occurring through root contact, proof of airborne dispersal was readily obtained. Experiments showed that conidia from sporodochia on pea tissue could be carried in air currents and dispersed in water splashes. Thus, like several other Fusaria (Cohen, 1942; Silveira, 1946; Pady, 1951; Rajan, Nigam & Shulka, 1952; Pady & Kapica, 1953) *F. solani* can, under suitable conditions, form part of the air-spora. It would be interesting to discover how far infection could be spread in this way in the field.

I have great pleasure in thanking Dr C. J. Hickman, Botany Department, University of Birmingham, where this work was carried out, for his help and criticism throughout.

REFERENCES

Bockman, H. (1950). Über Fusskrankheiten an Erbsen. *Z. PflKrankh.* **57**, 327–334.

Buxton, E. W. (1955). *Fusarium* diseases of peas. *Trans. Brit. mycol. Soc.* **38**, 309–316.

Cohen, V. L. (1942). The content of fungus spores in the air in Buffalo, New York. Abstr. in *J. Bact.* **43**, 115–116.

Conn, H. J. (1932). The Cholodny technic for the microscopic study of the soil microflora. *Zbl. Bakt.* (Abt. 2), **87**, 233–239.

Davies, D. L. G. (1943). Infection of pea seedlings with the *Fusarium* causing foot-rot, and some environmental relations of the fungus. *Rep. agric. hort. Sta. Bristol*, 1942, pp. 103–107.

Jones, F. R. (1923). Stem and rootrot of peas in the United States caused by species of *Fusarium*. *J. agric. Res.* **26**, 459–476.

Keen, B. A. & Raczkowski, H. (1921). The relation between the clay content and certain physical properties of a soil. *J. agric. Sci.* **11**, 441–449.

New South Wales (1943). Plant diseases. Notes contributed by the Biological Branch. *Agric. Gaz. N.S.W.* **54**, 116–120, 155–158.

New Zealand (1949). *Twenty-third Annual Report of the Department of Scientific and Industrial Research, New Zealand*, 88 pp.

Ogilvie, L. (1929). Observations on the diseases of market garden crops in the Vale of Evesham. *Rep. agric. hort. Res. Sta. Bristol*, 1928, pp. 127–146.

Padwick, G. W. (1938). Complex fungal rotting of pea seeds. *Ann. appl. Biol.* **25**, 100–114.

Pady, S. M. (1951). Fungi isolated from Arctic air in 1947. *Canad. J. Bot.* **29**, 46–56.

Pady, S. M. & Kapica, L. (1953). Air-borne fungi in the Arctic and other parts of Canada. *Canad. J. Bot.* **31**, 309–323.

Rajan, B. S. V., Nigam, S. S. & Shulka, R. K. (1952). A study of the atmospheric fungal flora at Kanpur. *Proc. Indian Acad. Sci.* **35**, 33–37.

Silveira, V. D. (1946). Flora microbiana do ar atmosférico. *Bol. Soc. brasil. agron.* **9**, 9–33.

Snyder, W. C. (1935). St John's Disease of pea in Europe. *Zbl. Bakt.* (Abt. 2), **91**, 449–459.

Togashi, K. (1928). Three *Fusaria* which cause the wilt disease of pea. *Jap. J. Bot.* **4**, 153–188.

Walker, J. C. & Snyder, W. C. (1933). Pea wilt and root rots. *Bull. Wis. agric. Exp. Sta.* no. 424, 1–16.

Went, J. C. (1934). *Fusarium*-aantastingen van Erwten. Thesis, University of Utrecht.

EXPLANATION OF PLATE 10

Fig. 1. Sporodochia of *Fusarium solani* on a portion of a longitudinal section of pea epicotyl. Preparation stained with acid fuchsin in lactophenol and mounted in lactophenol. × 90.

Fig. 2. The effect of infection by *F. solani* on the aerial parts of 6-week-old Early Bird peas inoculated with 'conidia' and grown at 60° F.

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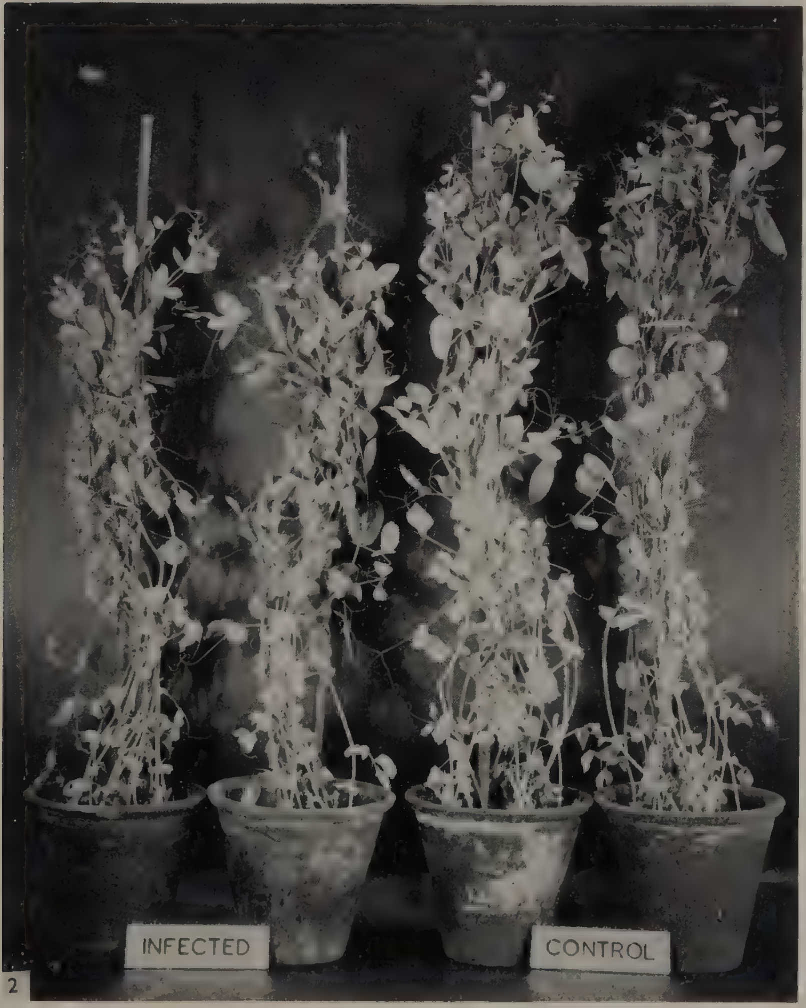

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(*Facing p.* 212)

Trans. Brit. mycol. Soc. **42** (2), 213–222 (1959).

SPORE SIZE IN RELATION TO CULTURE CONDITIONS

By C. N. WILLIAMS*

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(With 8 Text-figures)

Illumination, temperature, pH and nutrition were found to affect the spore size of *Mucor dispersus*, *Trichothecium roseum*, *Sordaria fimicola* and *Coprinus heptemerus*. Cultures grown in the dark (or at a low illumination in *C. heptemerus*) produced significantly larger spores than illuminated cultures.

Within limits, *S. fimicola* produced larger spores at higher temperatures, but the other organisms, larger spores at lower temperatures.

Variation in pH (using phosphate buffers) affected the spore size of all the species. *M. dispersus* and *C. heptemerus* produced larger spores on the more acid media, and *T. roseum* on the more alkaline. *S. fimicola* showed a minimum spore size at pH 6·4 and produced larger spores at higher and lower values.

The effect of nutrition was rather complex; *S. fimicola* produced smaller spores on media containing glucose in addition to cellulose than on media with cellulose as the only carbohydrate source. *M. dispersus* and *C. heptemerus* showed an increase in spore size with increasing sugar concentration. In *M. dispersus* it was unaffected by the concentration of organic N (asparagine) whilst in *C. heptemerus* it decreased at higher N (yeast) concentration.

Conidia of *T. roseum* on glucose/asparagine media at a low asparagine concentration (0·05 %) did not increase in size with increasing sugar concentration, but at high asparagine levels (0·2–1 %) a sharp increase occurred between 2 % and 5 % glucose. Using inorganic N ($(NH_4)_2SO_4$ and KNO_3) instead of asparagine, at correspondingly low and high concentrations, there was a regular increase in conidium size with increasing glucose at both levels.

The results presented suggest that spore size can be used to characterize species only if the environmental conditions are completely standardized.

INTRODUCTION

A review of the literature shows that external conditions affecting sporulation and fruit-body production in the fungi have been studied in a fair number of species, but the effects of these conditions on the size and form of the spores themselves have seldom been considered.

Temperature was studied by Thung (1926), who demonstrated that the size of conidia of *Peronospora parasitica* is less at 20° C. than at 5° C., and Nitimargi (1935), who showed that spore length in *Phomopsis coneglanensis* diminishes with temperatures above 20° C.

Although the effect of light on sporulation and fruit-body production has been investigated for a number of species, the effect of this factor on spore size has not received the same attention. However, Harter (1939), in three species of *Fusarium*, found that the conidia were significantly larger in cultures grown in the light than in those grown in the dark, and Snyder

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& Hansen (1941), working with ten strains of three species of *Fusarium*, confirmed Harter's results.

There is some evidence that variations in the medium affect spore size. Nitimargi (1935) reports that the proportions of the two types of conidia from species of *Cytosporina*, *Phomopsis* and *Diaporthe* varied from predominantly small oval to predominantly large curved as the concentration of glucose in the media was increased; also, the dimensions of the spores increased with glucose concentration. In contrast to this, an increase in asparagine induced the production of small oval conidia and decreased the length of both types.

The present work was undertaken to determine how the size of fungal spores is influenced by cultural conditions. A wide range of morphological types was chosen in the hope that some generalizations might be made about the effects of environmental factors.

GENERAL PROCEDURE

The fungi used, namely *Mucor dispersus* Hagem, *Sordaria fimicola* (Rob.) Ces. & De Not., *Trichothecium roseum* Link ex Fries, and *Coprinus heptemerus* M. Lange & Smith were chosen principally for the ease with which they could be grown in culture, the comparatively large size of the spores, and because they represented four morphologically distinct and diverse types. In three of these the spores are borne aerially, and in *S. fimicola* the ascospores are well protected by a substantial perithecial wall, so that in all four species the spores are free from the direct and immediate influence of the medium.

Preparatory to starting the investigations pure lines of each species were established either from single spores or from single hyphal tips.

The average spore length (calculated from 75 or 100 measurements) are used for comparison. In *M. dispersus* and *T. roseum* the spore populations on which measurements were made were obtained by mounting in water aerial growth from mature sporing cultures. In *S. fimicola* the black spore deposit discharged on to the lid of the Petri dish was sampled, and in *C. heptemerus* the spore prints from individual fruit-bodies.

In the first three fungi large numbers of sporing organs were sampled. The agaric *C. heptemerus*, however, produced only a small number of fruit-bodies, and the possibility of variations between those from the same culture could not be overlooked. In consequence, all maturing fruit-bodies were sampled separately and compared. The combined average of all the fruit-bodies from each culture was used to compare the effects of different treatments.

Spores for measurement were chosen at random by a standard procedure, the significance of the measured differences being tested by Student's '*t*' test. In the figures the significance of the difference between adjacent points on the graphs are shown in the following manner: P less than 0·1 (*), P less than 0·01 (**), P less than 0·001 (***), P greater than 0·1 (o).

LIGHT

With all organisms the source of illumination was a pair of 'daylight' fluorescent lamps. Light cultures were incubated at about 120 f.c.; corresponding dark cultures of *M. dispersus*, *T. roseum* and *S. fimicola* were enclosed in light-tight boxes. *C. heptemerus* would not sporulate in the absence of light, so a lower illumination of 60 f.c. was used to study the effect of decreasing the light.

All cultures were grown at 20° C., *M. dispersus* and *T. roseum* on 2 % malt agar, *S. fimicola* on yeast extract filter-paper agar (0·2 % 'Bacto', 2 % filter-paper) and *C. heptemerus* on a modification of the medium used by Bille-Hansen (1956)*.

All organisms produced larger spores in the dark (or at low illumination in *C. heptemerus*) than under conditions of strong illumination (see Table 1).

Table 1. *Average sizes, in μ, of spores from light and dark cultures of four fungi*

	M. disperus	*T. roseum*	*S. fimicola*	*C. heptemerus*
Light	16·9	14·0	21·2	13·1
Dark	18·0	14·6	22·3	14·3
Difference	1·1	0·6	1·1	1·2
Significance (*P*)	$<0·01$	$<0·1$	$<0·01$	$<0·001$

TEMPERATURE

In these experiments, all cultures except those of *C. heptemerus*, which was illuminated at 120 f.c., were grown in the dark and exposed to light only at times of inspection. Incubation temperatures were within the range 7–35° C. The culture media were the same as those used in the illumination experiments, except that for *T. roseum* two glucose asparagine media were substituted, one with high and the other with low glucose content.†

In general, *S. fimicola* produced larger spores at higher temperatures and the other organisms larger spores at lower temperatures (Fig. 1).

T. roseum and *C. heptemerus* show minimum spore sizes at 25 and 20° C., respectively, but otherwise the larger spores were produced at the lower temperatures. The differences are mostly significant.

T. roseum produced larger spores at the higher glucose concentration, but the two curves are parallel, probably because the effects of nutrition and temperature are additive.

pH

Media were buffered with phosphate mixtures ($Na_2HPO_4 . 12H_2O$ and KH_2PO_4) at a range of pH levels. The concentration of phosphates in the media was approximately 0·4 %. The actual pH values after autoclaving were reasonably close to the theoretical values. A liquid medium was used for *T. roseum*. With the others semi-liquid media containing 0·5 % agar were used, this being soft enough to insert the glass electrode of the pH meter.

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* 1 % Maltose, 0·003 % fresh yeast (dry basis), 0·05 % $MgSO_4 . 7H_2O$, 0·05 % $Ca(NO_3)_2$, made up with distilled water and buffered with a mixture of $Na_2HPO_4 . 12H_2O$ and KH_2PO_4 (*c.* 0·4 % combined) to pH 7·3.

† 2 % or 10 % glucose, asparagine; 0·5 % $MgSO_4 . 7H_2O$; 0·05 % potassium phosphate (0·1 % KH_2PO_4 and 0·1 % K_2HPO_4) made up with distilled water.

In *T. roseum*, the pH increased with growth in spite of the buffers, due presumably to the conversion of asparagine to ammonia. This tendency was counteracted by daily addition of N-HCl where necessary, a universal indicator (B.D.H.) being incorporated in the medium.

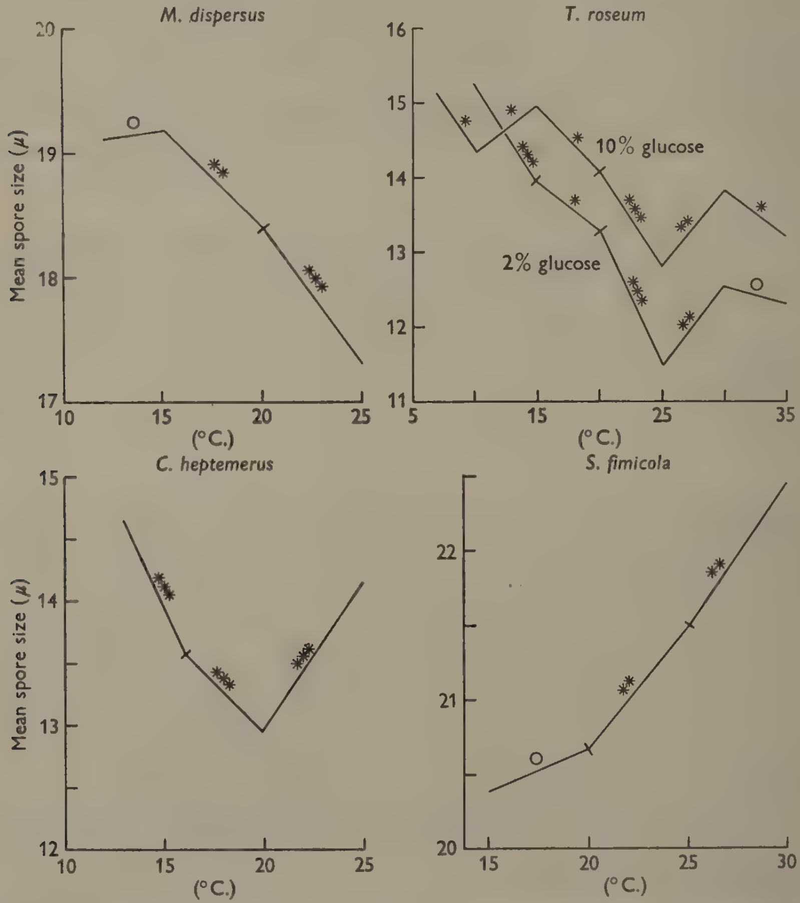

Fig. 1. Spore size in microns plotted against temperature for *M. dispersus*, *T. roseum*, *C. heptemerus* and *S. fimicola*. For *T. roseum* two media of different glucose concentrations were used. Significance of differences between neighbouring points on graphs: * = < 0·1; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$; o = $P > 0{\cdot}1$.

Except for the buffer mixture, the same nutrients were used to culture *S. fimicola* and *C. heptemerus* as in the preceding experiments. *M. dispersus* and *T. roseum* were grown on glucose-asparagine media, the former on a medium with 2 % glucose and 0·2 % asparagine, and the latter on one containing 5 % glucose and 0·2 % asparagine. In addition, 0·05 % $MgSO_4 . 7H_2O$

was added and the media were made up with distilled water. Experiments were carried out at 20° C. in the dark, except for *C. heptemerus* which again was illuminated at 120 f.c.

M. dispersus and *C. heptemerus* produced significantly larger spores at lower pH, but *T. roseum* produced larger spores at the higher pH levels. *S. fimicola* showed a minimum spore size at pH 6·3. The significance of these differences was variable (see Fig. 2). *C. heptemerus* sporulated at two pH levels only, but the difference between the spores produced at these levels was highly significant ($P < 0{\cdot}001$).

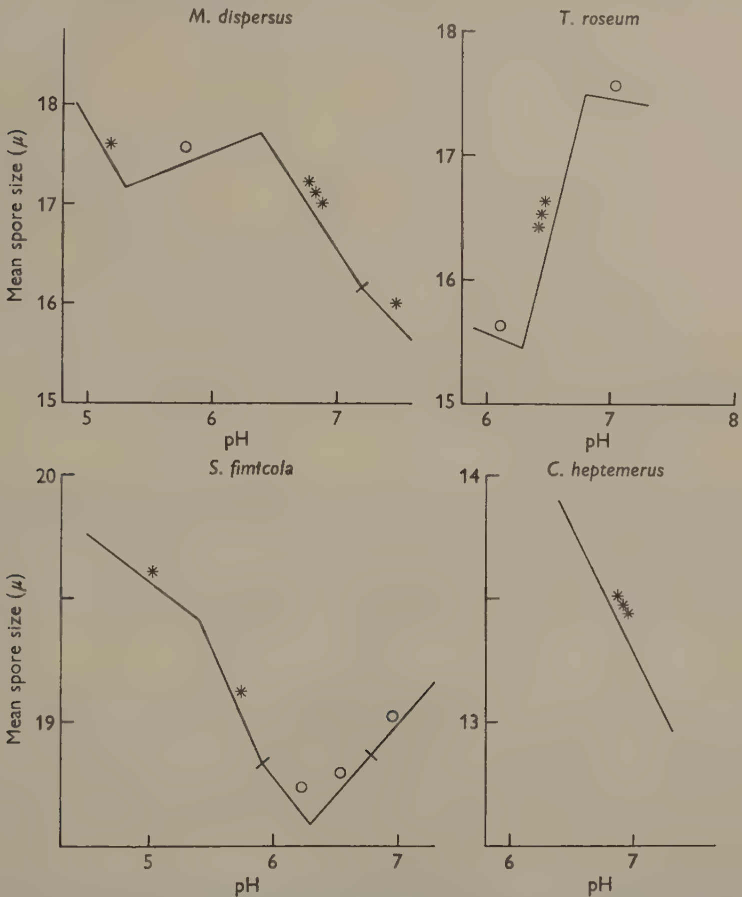

Fig. 2. Spore size in microns plotted against pH for *M. dispersus*, *T. roseum*, *S. fimicola* and *C. heptemerus*. Significance of differences between neighbouring points on graphs: * = $P < 0{\cdot}1$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$; o = $P > 0{\cdot}1$.

NUTRITION

The effect of nutrition on the spore size of the different fungi was rather variable and they will be considered species by species. All experiments were carried out at 20° C. in the dark, except for *C. heptemerus* which again was illuminated at 120 f.c.

Mucor dispersus. Five different media containing respectively, 0·2, 1, 2, 5 and 10 % malt extract in 2 % agar were used. There was a significant increase in spore size with increasing concentration up to 5 % (Fig. 3).

Having established that spore size was affected by the concentration of malt in the medium, it was decided to see if the effect was due to carbohydrate or to organic nitrogen supply. Thus, varying amounts of glucose and asparagine (0, 2 and 10 % glucose, and 0·05, 0·2 and 0·5 % asparagine) were added to a basic medium containing phosphate (0·1 % KH_2PO_4 and 0·1 % K_2HPO_4), 0·05 % $MgSO_4 . 7H_2O$, 2 % agar and tap water. The organic constituents were used in all possible combinations.

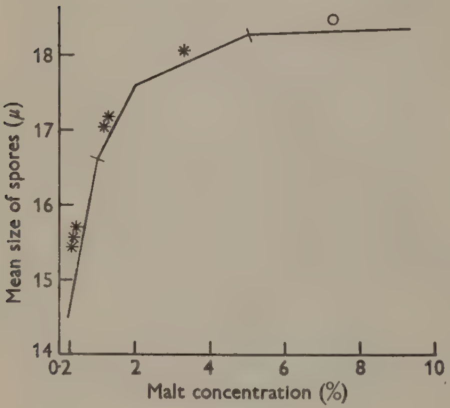

Fig. 3

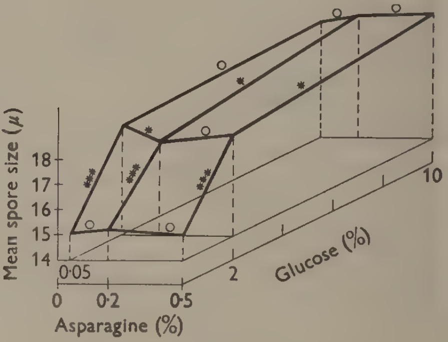

Fig. 4

Fig. 3. *M. dispersus.* Spore size plotted against malt concentration in medium. Significance of differences between neighbouring points on the graph: * = $P < 0{\cdot}1$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$; o = $P > 0{\cdot}1$.

Fig. 4. *M. dispersus.* Effect of glucose and asparagine concentrations on spore size. Significance of differences between neighbouring points on graphs: * = $P < 0{\cdot}1$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$; o = $P > 0{\cdot}1$.

It is clear (Fig. 4) that higher glucose concentrations produce larger spores, but varying the asparagine concentration has little effect.

Increasing sucrose also results in significant increase in spore size, but the spores are smaller than those produced on glucose media of the same concentration (Fig. 5). If the molarities of the two sugars are plotted on the horizontal axis instead of the percentage concentration, the difference between the two curves is lessened.

Trichothecium roseum. To study the effect of glucose and asparagine on the conidium length, glucose concentrations of 0, 2, 10 and 15 % were used in combination with asparagine at 0·05, 0·2, 0·5 and 1 % levels. The inorganic constituents were the same as for *M. dispersus.* The results (Fig. 6) show clearly that in contrast to *M. dispersus* the concentration of asparagine, as well as that of glucose, affects the mean length of the conidia. There is a striking difference between the glucose series containing 0·05 % asparagine and those of higher asparagine content. In the latter there is a sharp increase in size between the 2 % and 10 % glucose levels, but at the lowest asparagine level this was not observed. Furthermore, at the lower glucose concentrations (0 and 2 %) conidium size was affected inversely by asparagine concentration, and there was a decrease in conidium size between the 0 and 2 % glucose levels. Further experiments confirmed these results and showed that conidium size increases in an even sharper manner between 2 and 5 % glucose with high asparagine.

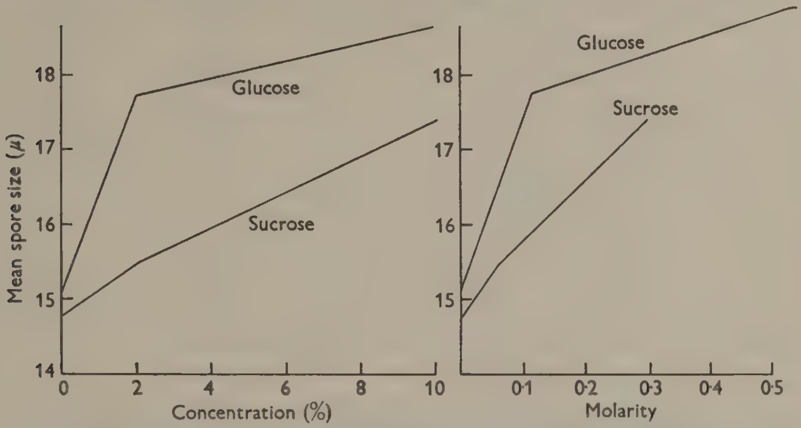

Fig. 5. *M. dispersus.* Spore size plotted against sugar concentration (left) and sugar molarity (right).

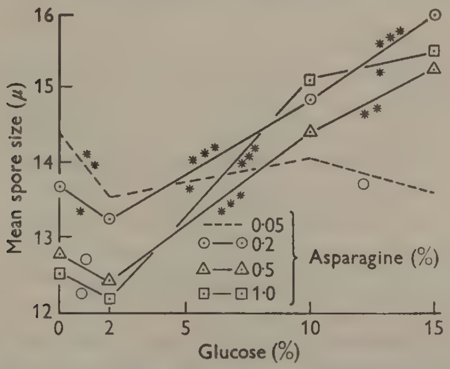

Fig. 6. *T. roseum.* Spore size plotted against glucose concentration at various levels of asparagine supply. Significance of differences between neighbouring points on the graphs: * = $P < 0{\cdot}1$; ** = $P < 0{\cdot}01$; *** = $P < 0{\cdot}001$; o = $P > 0{\cdot}1$.

The pH of the cultures increased considerably with growth of this organism, particularly on media without glucose. This possibly explains the relatively large size of the spores on media without glucose, compared with those containing 2 % glucose, as it was seen in earlier experiments that larger spores were produced on the more alkaline media.

Inorganic nitrogen in place of asparagine. It was thought that the difference in form of the curves relating spore size to glucose concentration at low and high levels of asparagine might be associated with the utilization of asparagine as a source of energy. Where the supply of amino acid to the fungus is low, and possibly limiting, this oxidation could perhaps lead to the unexpected response of spore size to glucose concentration observed at the low asparagine level. To test this theory two inorganic N sources (KNO_3 and $(NH_4)_2SO_4$) were used instead of asparagine. Only the critical concentrations were studied, namely, 2 and 5 % glucose, and N equivalents of 0·05 and 0·5 % asparagine.

The results (Fig. 7) show no difference in behaviour at the low and high N levels, there being an increase in spore size with glucose at both.

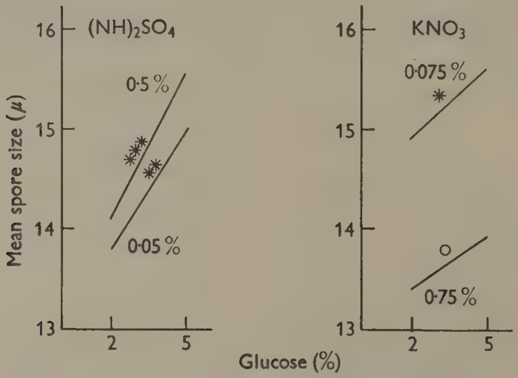

Fig. 7. *T. roseum*. Effect of different concentrations of inorganic nitrogen on spore size at two different glucose levels. Significance of differences between neighbouring points on the graphs: * = $P < 0·1$; ** = $P < 0·01$; *** = $P < 0·001$; o = $P > 0·1$.

Sordaria fimicola. There was a fundamental difference between the nutritional studies of *S. fimicola* and those of the preceding two organisms. This fungus could be induced to sporulate and to discharge spores only on media containing cellulose, the addition of soluble sugars merely providing an additional source of carbohydrate. The basic medium used in these experiments was 2 % filter-paper agar containing varying amounts of 'Bacto' yeast extract.

The addition of large quantities of glucose (5–10 %) prevented sporulation completely, but it occurred, somewhat delayed, with concentrations up to 2 %. The concentrations used in the experiments were: 0, 0·5, 1 and 1·5 % glucose. The N was varied by adding either 0·2 or 0·5 % 'Bacto' yeast extract.

It is seen (Fig. 8) that the spores are significantly larger ($P < 0·01$) on the medium containing cellulose as the only carbohydrate than on those with additional sugars. Variations in the amount of glucose or 'Bacto' added produced no significant difference. Vegetative growth, however, appeared to be more vigorous on the media containing glucose.

Coprinus heptemerus. Bille-Hansen's medium was used to study the effect of nutrition, but the concentrations of maltose and yeast-extract were varied. The yeast-extract was prepared by autoclaving fresh yeast and centrifuging clear. (In previous experiments whole yeast was used.)

All fruit-bodies were sampled separately. Variations occurred between fruit-bodies from the same cultures, but those were small compared with the variations between treatments.

The results (Table 2) indicate relatively large spore size with high maltose and small spore size with high yeast extract. A further experiment, in which whole yeast was substituted for yeast extract, fully confirmed these results.

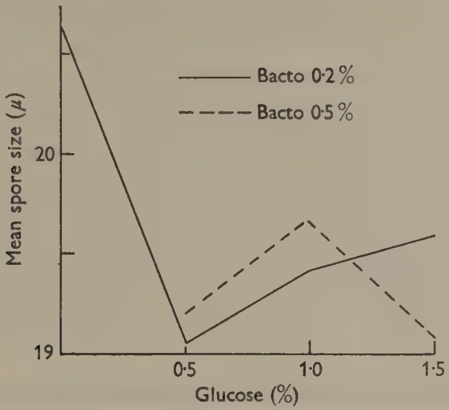

Fig. 8. *S. fimicola.* Effect of glucose and 'Bacto' yeast extract at two levels on spore size in filter-paper medium

Table 2. *The effect of maltose and yeast-extract concentration on* Coprinus heptemerus

Maltose (%)	Yeast extract (D basis)	Sporulation time (days)	Number of mature fruit-bodies	Average spore size (μ)
0·2	0·008	∞	0	—
1	0·008	17	4	12·84
3	0·008	19	3	13·69
1	0·004	18	4	14·09
1	0·0016	∞	0	—

DISCUSSION

The results of this investigation show clearly that, for the fungi studied, almost any change in nutrition, or in other environmental conditions, leads to a significant, and often quite large, change in spore size.

When details are considered, it is found that the effect of a particular factor on spore size may be quite different for different kinds of fungi. Thus, while soluble carbohydrate increases spore size in *M. dispersus* and *C. heptemerus*, it decreases it in *S. fimicola*; spore size in *T. roseum* increases with glucose only at high asparagine level. Similarly, higher temperatures favour large spores in *S. fimicola*, but reduce, in general, the spore size of the other fungi. The effect of pH is also variable, *M. dispersus* and *C. heptemerus* producing larger spores at lower pH levels, and *T. roseum* larger spores at higher pH. The only factor that affected spore in the same direction in all the fungi studied was light, the spores being larger at lower illuminations.

The fact that this character is so readily altered has a considerable bearing on taxonomy since it is frequently used to separate taxa.

I wish to thank Prof. C. T. Ingold for suggesting and directing the course of this research.

REFERENCES

Bille-Hansen, E. (1956). The nitrogen requirements of *Coprinus heptemerus* M. Lange and Smith. *Saertryk af Friesia*, **5**, 3–5, 190–195.

Cesati, V. & De Notaris, G. (1863). Schema di classificazione degli sferiacei italici. *Comm. Soc. Crit. Ital.* **1**, 225–227, 234.

Hagem, O. (1910). Neue Unterschungen über Norwegische Mucorineen. *Ann. Mycol., Berl.*, **8**, 265–286.

Harter, L. L. (1939). Influence of light on the length of the conidia in certain species of *Fusarium*. *Amer. J. Bot.* **26**, 234–243.

Lange, M. & Smith, A. H. (1953). The *Coprinus ephemerus* group. *Mycologia*, **45**, 747–780.

Link, F. (1809). Ordines plantarum naturales. *Mag. Gesell. Natur. Fr.* **3**, 4–42.

Nitimargi, N. M. (1935). Studies in the genera *Cytosporina*, *Phomopsis* and *Diaporthe*. VII. Chemical factors influencing sporing characters. *Ann. Bot., Lond.*, **49**, 19–40.

Snyder, W. C. & Hansen, H. N. (1941). The effect of light on the taxonomic characters of *Fusarium*. *Mycologia*, **33**, 580–591.

Thung, T. H. (1926). Opmerkingen over *Peronospora parasitica* op Kool (Bemerkungen über die Kohl-*Peronospora*). *Tijdschr. Plziekt.* **32**, 161–179.

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CONTRIBUTIONS TOWARDS A MONOGRAPH OF THE GENUS *PLUTEUS*. II.

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(With 4 Text-figures)

Some data on type material of the *Pluteus* species not indicated specifically as such in the past, and others which have been misinterpreted, are here provided and discussed. The species analysed are: *P. granulatus* Bres., *P. griseibrunneus* Murr., *P. longipes* Murr., *P. murinus* Bres., all belonging to the European and North American mycoflora.

In the first part of my studies on the genus *Pluteus* (these *Transactions*, **39**, 145–232, 1956) some species described in this genus were omitted because, at the time, material was not readily available for re-examination. The present paper is intended to provide data on some of these neglected species so that their precise position can be ascertained.

PLUTEUS GRANULATUS Bres., *Fungi Trid.* 1, 10, 1881 (Fig. 1).

Authentic material from the same general region (Mendola, on conifer trunks, Aug. 1904, leg. & det. Bresadola, **NY**) as the type collection has been analysed.

Spores 7–8·8 × 6–7 μ, subglobose to short cylindrical, not globose. *Basidia* 22–31 × 8·8–11 μ, variable in shape, ventricose-subclavate, some shaped like the 'Urnigera basidia', 4-spored. *Cystidia* on edges and sides of lamellae similar, only more numerous on edge, all hyaline, 50–78 × 7·7–13·2 μ, often short pedicellate, apex 3·3–6 μ diam. and frequently subcapitate (4·5–6 μ diam.) or again attenuated above (to 3·7–4·5 μ diam.) and in this case also frequently subcapitate, the upper portion in many cases more or less irregular, flexuous or asymmetrical, some somewhat melleous but not strongly incrusted, always thin-walled. *Epicutis* of repent voluminous elements which are strictly elongated and reach 20–25 μ diam., yellowish hyaline to yellow, with broadly rounded tips. Among these hyphae some fascicles of shorter elements break through which are somewhat agglutinated to each other, winding or curved, often with constrictions below a broadly capitate apex, so that they may appear to be sphaerocysts, others in reality so shortened that they are nearly balloon shaped, succineous to deep yellow, 16–50 × 11–16·5 μ, some with irregularly sinuate, but all with very broadly rounded tips. All hyphae with clamp connexions. The fascicles described correspond to the granules of the pileus seen macroscopically.

The structure of the epicutis of the pileus might suggest the structure of a representative of the sect. Celluloderma, subsect. Mixtini. However, two reasons are in favour of maintaining this species in the sect. Trichoderma Fayod (sensu lato as used temporarily in the preceding paper), subsect. Hispidodermini (or, if an autonomous section is admitted for this group: sect. Hispidoderma Fayod): (1) the fact that the species has no relatives in subsect. Mixtini; (2) the fact that the overall structure of the pileus covering is that of a Hispidoderma and only the fascicles, erumpent from the longitudinal large hyphae of the epicutis, have a 'Mixtini-structure' to a certain degree, although even here the isodiametric appearance of some elements is in reality caused by constrictions of elongated elements and by the angle of observation (since the flexuous elements often show their obtuse apex so that it is necessarily seen from above). The presence of a small number of exceptionally short elements in the epicutis is, in Hispidoderma, not exactly unique.

Romagnesi (*Bull. Soc. mycol. Fr.* **72**, 218, 1956) describes a fungus under the name of *P. granulatus* Bres. which seems to be very close to the material revised by me, but differs in being larger, having smaller spores, lacking short elements in the granules, and having a firm, solid (instead of fistulose) stipe.

It seems, then, that *P. granulatus* Bres. sensu Kühner & Romagnesi is at least a variety of the typical *P. granulatus* as represented by the authentic material analysed above.

PLUTEUS GRISEIBRUNNEUS Murr., *N. Amer. Flora*, **10** (2), 133, 1917 (Fig. 2)

The type collected by Earle in the City Park of New Orleans, no. 71, 6 Sept. 1908 (**NY**) gave the following results:

Spores 5·5–6 × 4·2–4·5 μ, ellipsoid. *Cheilocystidia* hyaline, subvesiculose, mostly collapsed in the type. *Pleurocystidia* not pigmented, not or not strongly incrusted, collapsed. *Epicutis* of pileus in the centre of the pileus—a trichodermium, but not now visibly palisadic, consisting of elongated hyphae, the terminal members 65–120 × 7·7–14·2 μ, light fuscous from an intracellular, evenly dissolved or sometimes locally condensed pigment, thin-walled, with broadly rounded tip, cylindrical or ampullaceous-elongate, or cylindrical with a constriction, apical portion and constricted portion about 6·7–8 μ diam. All hyphae without clamp connexions.

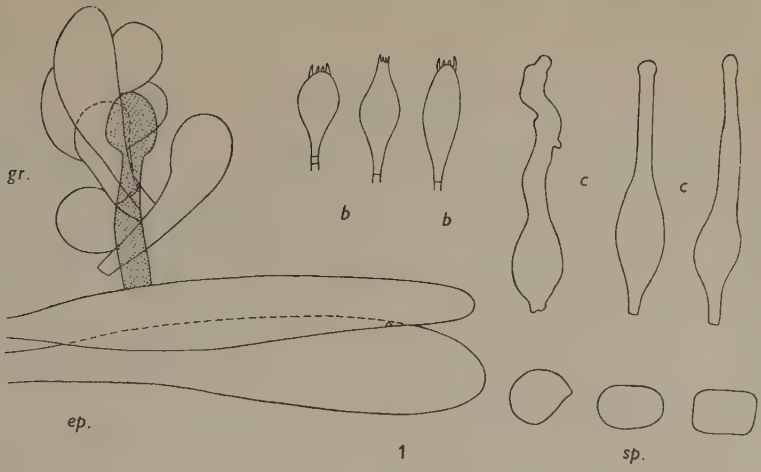
This is apparently a good species. I do not know it in the fresh state. In the key to the species of its group (Singer, *loc. cit.*, key V, p. 228) it would key out with *P. floridanus* Murr. ex Sing. It differs, however, from *P. floridanus* in having considerably larger carpophores, more elongate spores (mostly over 1 μ longer than broad in *P. griseibrunneus*, and less than 1 μ longer than broad in *P. floridanus*).

PLUTEUS LONGIPES Murr., *N. Amer. Flora*, **10** (2), 133, 1917 (Fig. 3)

Pluteus whiteae Murr., *N. Amer. Flora*, **10** (2), 132, 1917

The type of *P. longipes* is deposited as *P. longipes* Earle but is the specimen cited in *North American Flora*. It comes from Redding, Conn., and was collected by F. S. Earle, no. 628, 22 July 1902 (**NY**). Its analysis showed that it was specifically identical with *P. whiteae*:

Spores 6–7·7 × 5–5·8 μ, short ellipsoid to subglobose. *Basidia* 23–28·5 × 9·4 μ, 4-spored. *Cheilocystidia* and *pleurocystidia* similar, except that



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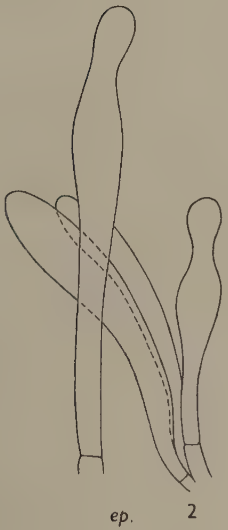

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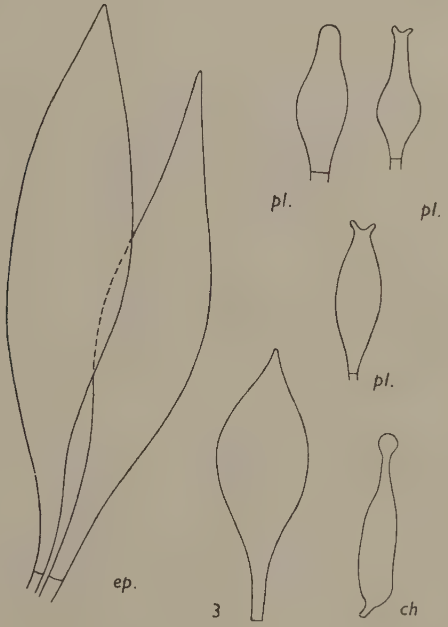

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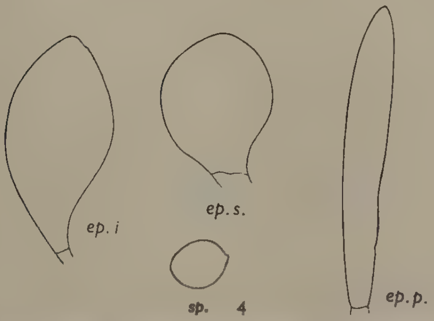

4

Fig. 1. *Pluteus granulatus* Bres., authentic material; *gr.*, structure of the granules of the pileus; *ep.*, elements of the epicutis of the pileus between the granules; *b.*, basidia; *c.*, cystidia (all × 500); *sp.*, spores, × 1000.

Fig. 2. *P. griseibrunneus* Murr., type material; *ep.*, elements of the epicutis of the pileus, × 500.

Fig. 3. *P. longipes* Murr., type material; *ep.*, cystidioid elements of the epicutis of the pileus, and *pl.*, pleurocystidia, *ch.*, cheilocystidia, all × 500.

Fig. 4. *P. murinus* Bres., type material; *ep.i.*, epicuticular elements, intermediate type; *ep.s.*, epicuticular element, sphaerocyst; *ep.p.*, epicuticular element, pilose type, all × 500; *sp.*, spore, × 1000.

the former are more numerous. *Cystidia* 30–80 × 7·7–18·7 μ, versiform, most frequently broadly ventricose below, to ampullaceous, mucro or neck varying from insignificant to rather long and in the latter case usually with two horn-like (but mostly obtuse) appendages, pale melleous refringent opaque in places, apparently with a thin, closely applicate resinous incrustation. *Epicutis* of pileus characteristically of voluminous strongly elongated elements, 65–152 × 12·5–31 μ, with a very dilute melleous-brownish pigment, hyaline, ventricose, appressed, characteristically attenuate and often subacute at the tip and therefore somewhat like those of *P. fuliginosus*. Clamp connexions: none seen.

According to Art. 57 of the International Code, *P. longipes* Murr. is here accepted as the valid name for this North American species. A full description of it was given by Singer in 1956 (*loc. cit.*) under the name *P. whiteae*.

PLUTEUS MURINUS Bres., *Ann. Mycol., Berl.*, **3**, 160, 1905 (Fig. 4)

What is evidently part of the type collection, Gocciadoro (near Trento), Leg. & det. Bresadola, October 1903 (**NY**) was analysed in order to compare the data thus obtained with the descriptive data available from collections made by Kühner & Romagnesi on one hand, and L. N. Vassilieva (det. Singer) on the other. The result is the following:

Spores 6·3–7·7 × 4·6–6·7 μ, subglobose to short ellipsoid, never globose. *Basidia* 4-spored. *Cystidia* neither metuloid nor coloured. *Epicutis* of the pileus of dimorphic elements, some distinctly subisodiametric, e.g. 33–40 × 25–30 μ, some elongated; the elongated ones either cystidioid and relatively broad, e.g. 50–60 × 22–29 μ, or almost pilose, e.g. 82–83 × 11·5–12·5 μ. All elements of the *epicutis* very broadly rounded, with a light fuscous intracellular pigment, which is evenly dissolved (without condensations). All hyphae without clamp connexions.

The type specimen seen corresponds well to the illustration given by Bresadola (*Inconogr. Mycol.* **11**, pl. 533, 1929), but seems to have a slight bulb. The disc is now somewhat umbonate in the dried material and entire, not crevassed except in the outer (larger) portion of pileus where the surface is radially rimulose showing the underlying trama. Habitat: earth, in meadows.

I cannot guarantee that either Romagnesi's material or my own had sphaerocysts in the covering layer of the pileus. The description given by Romagnesi (*Rev. Mycol., Paris*, **2**, 134, 1937) does not suggest it, nor do my own notes mention dimorphic structure. Furthermore, my own notes refer to '*Pluteus murinus* (var. ?)' and Romagnesi described the lamellae as cream, then rose colour, while Bresadola said 'ex albo carneae'. It is quite possible that the new interpretations of the species are not identical with the type.

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FACTORS AFFECTING THE INVASION OF TOMATO ROOTS BY *VERTICILLIUM ALBO-ATRUM*

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(With Plates 11 and 12)

Anatomical studies have been made of the process of invasion of seedling tomato roots in sterile nutrient solutions and in non-sterile John Innes compost 2 weeks after inoculation with *Verticillium albo-atrum*. Three levels of sucrose, 200, 2000 and 20,000 p.p.m., were supplied in the sterile solutions. With 200 p.p.m. the fungus was confined to the piliferous layer and lignitubers projected into the outer layer of cortical cells. With 2000 p.p.m. sucrose, invasion of the outer layers of the cortex and lateral root tips occurred and large numbers of lignitubers were found in this region. Invasion of all root tissues, including the xylem, occurred with 20,000 p.p.m. sucrose and few and small lignitubers were evident. Nitrogen was supplied as nitrate at 1·58, 15·8 and 158 p.p.m. in sterile solutions but did not affect the degree of penetration by hyphae or lignituber production. In non-sterile compost or sand the degree of invasion and lignituber production were comparable with those found with 200 p.p.m. sucrose in sterile nutrient solution.

Root damage induced by transplanting or by deliberate scalpel cuts resulted in rapid systemic invasion by the fungus. Watering conidial suspensions onto soil containing undamaged roots did not usually result in systemic infection.

Introduction

As a preliminary to studying the influence of environmental factors on infection of tomato by *Verticillium albo-atrum*, information was sought concerning the initial stages of the process. Van der Meer (1925) studied the invasion of potato and cucumber roots by this fungus and concluded that intact roots could be entered either through 'epidermal' cells or through the root hairs. Hyphae eventually penetrated the thin walls of the young xylem vessels. Her anatomical studies lent support to the earlier results of Bewley (1922) who concluded, from sterile culture work, that this fungus could infect unwounded tomato roots. Similar results were reported by Isaac (1946) from a study of the invasion of sainfoin radicles by *V. dahliae* on Dox agar under sterile conditions. He reported deep penetration of all regions of the root 9 days after inoculation, and concluded that wounds were not necessary for infection to occur.

Derbyshire (1956) studied the invasion of tomato radicles by *V. albo-atrum* in sterile culture. Microtome sections of radicle tips cut 7–10 days after inoculation demonstrated penetration to the stele. Thus there might appear to be good evidence that *Verticillium* enters undamaged roots freely. However, studies of this kind have usually been carried out under conditions exceptionally favourable to the fungus and unfavourable to the host, as for example in the supply of large amounts of sugar in the medium.

Although free penetration of roots has been reported, the presence of 'conic protuberances' or 'lignitubers' has been noted from time to time in infected roots (van der Meer, 1925; Dufrénoy, 1927; Derbyshire, 1956; Talboys, 1958). The significance of these structures has not been fully evaluated, but with *V. albo-atrum* in hop, Talboys (1958) reported that lignituber frequency varied with the sensitivity of the host and the virulence of the pathogen. Endodermal resistance was also noted in hop, but this has not apparently been observed in tomato. Evidently conditions can exist under which invasion of roots by *Verticillium* may be circumscribed.

An attempt has been made in the present work to study the invasion pattern in tomato roots growing under various conditions.

EXPERIMENTAL METHODS

Inoculum

V. albo-atrum was isolated from a wilted tomato plant and cultured on potato-dextrose agar and on 3 % cornmeal-sand. Dark torulose hyphae were produced on both media, and conidia ($3–10 \times 1{\cdot}5–4\,\mu$) were freely formed. Conidial suspensions were prepared from 2-week cultures on potato-dextrose agar and adjusted to contain *c.* 12×10^6 conidia/ml. Inoculum used for the study of root infection in John Innes compost was taken from 3-week cultures on 3 % cornmeal-sand.

Sterile water culture

Tomato seeds were surface-sterilized by immersion for 20 min. in a freshly prepared filtrate of 10 % calcium hypochlorite solution, washed in sterile water and incubated for 4 days at 25° C. on damp filter pads in Petri dishes. The germinated seeds were planted on glass wool in 120 ml. sterile nutrient solution (Selman & Grant, 1957) in 1 lb. jam-jars, five seeds to each jar. The jars were lined with the glass wool and covered with Copenhagen-tank cups plugged with cotton-wool.

Seedlings were inoculated at the cotyledon stage by pipetting 1 ml. of the conidial suspension into each jar. Controls received 1 ml. of distilled water. At the same time sterile sucrose solution was added to the jars and the concentration adjusted as required.

Non-sterile soil cultures

A layer of cornmeal-sand inoculum, 1 in. thick, was placed on John Innes compost in 5 in. pots. The inoculum was lightly covered with compost, in which six germinated tomato seeds were planted. The radicles were thus free to grow into the inoculum. Control seedlings were grown in compost containing uninoculated cornmeal sand.

Examination of roots

Microscopical examination of roots was made by two methods. Root systems were cleared by immersion for 24 hr. in acidified chloral hydrate, dipped in cotton blue in lactophenol, mounted in dilute glycerine and viewed entire.

For wax embedding and sectioning, roots were preserved in formal-acetic alcohol. Cedar wood oil was used as a wax solvent and the procedure recommended by Johannsen (1940) was used. Sections, 10 μ thick, were stained with safranin and counterstained with cotton blue in 95 % alcohol.

INVASION OF ROOTS BY *VERTICILLIUM ALBO-ATRUM* IN A STERILE NUTRIENT SOLUTION

Transverse sections of infected roots were examined after 2 weeks growth in a glasshouse in sterile water culture containing 20,000 p.p.m. sucrose, in which the fungus was growing freely. Complete colonization of the cortex had occurred (Pl. 11, fig. 1). A few small lignitubers, 2–5 μ long × 3 μ wide at the base, were produced on the walls separating the piliferous layer from the outer cortical cells but hyphae were frequently seen to be growing out of them (Pl. 11, fig. 2). Hyphae penetrated the inner cortical cells freely, but lignitubers were rarely seen in these cells. The cytoplasm of these infected cells appeared granular and the walls stained red with safranin, but there was no evidence of changes occurring in advance of the fungus. Intercellular spaces in the infected region were frequently occluded and often contained safranin-staining globules.

Passage of the fungus across the cortex was mainly intracellular, although some colonisation of the intercellular spaces was observed. No appressoria were found, but the hyphae appeared constricted in passing through the walls of the cortical cells. The endodermis and pericycle were penetrated and hyphae eventually entered the primary xylem. The invading mycelium was invariably in the hyaline form.

INVASION OF ROOTS BY *VERTICILLIUM ALBO-ATRUM* IN JOHN INNES COMPOST AND NON-STERILE SAND

Sections of infected roots taken from seedlings grown for 2 weeks in John Innes compost showed that the piliferous layer and the intercellular spaces between this layer and the outer cortical cells were invaded. The fungus was not found in the cortex or within the stele. Entry of the piliferous layer was found to be through the cells from which root hairs had arisen and not through the walls of the hairs themselves. Root hairs were well developed on cells filled with hyphae, which might indicate invasion of the piliferous layer *after* the formation of hairs.

Lignitubers were found projecting into the cells of the outer cortical layer from the outer walls, particularly those bounding the intercellular spaces. The nuclei of the outer cortical cells were in close proximity to the lignitubers, but no other abnormality of the host cells was evident. There was no indication of the entry of hyphae by way of ruptured cortical cells where lateral roots had emerged. Attempts were made to establish the chemical nature of the lignituber walls but microchemical tests for lignin, suberin and cellulose were all negative. Sections of roots taken from infected plants grown for 7 weeks in non-sterile sand were similar but contained rather more and larger lignitubers, often exceeding 20 μ in length (Pl. 11, figs. 3 and 4).

EFFECTS OF NITROGEN AND SUCROSE ON INVASION

The above results suggested that the degree of invasion might be influenced by the conditions under which both host and parasite were developing. Experiments were therefore made to investigate some of the factors which might be expected to influence the process. Growth of the fungus *in vitro* was known to be greatly affected by the supply of sucrose, a factor which may be of prime importance in determining the 'inoculum potential' of a parasitic fungus. The supply of mineral nutrients may also affect the growth of both host and parasite, and, of the nutrient elements, the level of nitrogen has been found to be closely correlated with the incidence of *Verticillium* infection of tomato plants by Roberts (1943) and others. Sucrose and N supply were therefore selected for study.

Two experiments were made in sterile water culture. Differential levels of N were supplied from the time of germination of the tomato seed until sampling 3 weeks later and sucrose treatments were applied at the time of inoculation 2 weeks before sampling. Two foliage leaves were present on the plants at sampling.

Experiment 1. Three levels of N (1·58, 15·8 and 158 p.p.m.) were supplied as calcium nitrate and the level of Ca maintained by the addition of calcium chloride. There were four jars, with five seedlings per jar, in each nitrogen treatment; two jars were inoculated in each series. Sucrose was supplied at 200 p.p.m. and nutrients, other than N, were as given in the standard solution (Selman & Grant, 1957).

N supply did not visibly affect seedling growth. The roots of plants in all treatments, including those inoculated, were white, with well-developed laterals. Inoculated plants were slightly smaller than controls.

Microscopical examination showed that the growth of the fungus on the surface and in the piliferous layer of inoculated roots was not affected by N supply. With all treatments, penetration of the root was limited to the piliferous layer and lignitubers projected into the outer cortical cells. Counts were made of lignitubers in cleared preparations but no effect of N supply could be shown. Uninoculated control roots showed neither hyphae nor lignitubers.

Experiment 2. Two levels of N (15·8 and 158 p.p.m.) were supplied at each of three levels of sucrose (200, 2000 and 20,000 p.p.m.). There were four jars with five seedlings each per nutrient treatment; two jars were inoculated in each series. No effect of N supply on invasion could be discerned. Uninoculated seedlings were not visibly affected by the level of sucrose, whereas inoculated seedlings grown with 2000 or 20,000 p.p.m. were stunted, the cotyledons were yellow and wilted and the hypocotyls showed purple pigmentation. A large number of short roots, both lateral and adventitious, appeared on the inoculated plants receiving the two higher levels of sucrose, together with brown necrotic areas on the roots and on the base of the hypocotyl. Roots of inoculated seedlings receiving 200 p.p.m. sucrose appeared normal.

In the inoculated solutions, growth of the fungus increased with increasing sucrose concentration. With 200 p.p.m. sucrose, cleared roots appeared similar to those described in Exp. 1 and few lignitubers occurred (Pl. 12, fig. 5). With 2000 p.p.m. mycelial growth was denser and large numbers of lignitubers were observed in the outer layers of the cortex (Pl. 12, fig. 6). With 20,000 p.p.m. the roots were so densely covered with hyphae that it was impossible to discern lignitubers in cleared preparations. At the higher levels of sucrose, root hairs were frequently full of hyphae.

The extent of invasion of the roots was also greatly affected by sucrose level. With 200 p.p.m., the fungus was confined to the piliferous layer as in Exp. 1 (Pl. 12, fig. 7). Longitudinal sections of root tips showed that the meristem region was not invaded, though hyphae were seen in the root caps. With 2000 p.p.m. sucrose, the three outer layers of the cortex of the primary root were invaded. Cortical cells internal to the invaded region showed no abnormality. Slightly deeper penetration of the primary root was noted where lateral roots had emerged. Longitudinal sections of lateral root tips often showed deep penetration with complete colonization of the stele. Invasion of roots receiving 20,000 p.p.m. sucrose has already been described.

EFFECT OF MECHANICAL DAMAGE ON INFECTION

From the studies made in soil and in sterile water culture with low sucrose, it appeared that invasion of the cortex of intact roots did not immediately follow penetration of the piliferous layer. Since Selman & Pegg (1957) found that young tomato plants will become systemically infected 3 weeks after planting in soil with the roots in contact with agar inoculum discs, the possibility of rapid invasion through damaged tissues could not be discounted. An experiment was therefore made to study the effect of transplanting and deliberate cutting of the roots *in situ*.

Experiment 3. *Effect of transplanting or of cutting the roots on invasion by the fungus*. Tomato seeds, cultivar Potentate, were germinated on moist filter-paper and planted in soil blocks of John Innes compost. The blocks were placed in 5 in. pots and packed with loose soil before roots had appeared on their outer surfaces. The plants were raised in a glasshouse during June and July. Three weeks after germination the following treatments were applied (five plants per treatment).

(1) Plants were removed from the soil block and the roots were washed and dipped in a conidial suspension of *V. albo-atrum* before replanting.

(2) Plants were removed from the soil block and the roots were washed and replanted; 20 ml. conidial suspension was immediately poured over the soil surface.

(3) Plants were *not* removed from the soil block but all the roots running into the top 3 in. of loose soil were cut with a scalpel; 20 ml. conidial suspension was immediately poured over the soil surface.

(4) Plants were *not* removed from the soil block, but 20 ml. conidial suspension was poured over the soil surface.

Two weeks after inoculation the number of plants showing wilting symptoms was recorded. The plants were then sectioned at the cotyledonary node, using a freezing microtome, and five sections of each stem were examined in lactophenol and cotton blue. A count was made of the number of vessels containing hyphae. The results are summarized in Table 1.

Table 1. *Effect of root injury on infection*

Type of injury	Mode of inoculation	No. plants wilting 2 wk. after inoculation (out of 5)	No. plants with hyphae at cotyledonary node (out of 5)	Total no. vessels with hyphae (25 sections)	Mean no. vessels with hyphae per section
Transplanting	Roots dipped	3	5	416	16·7
Transplanting	Conidia poured	4	5	398	16·0
Scalpel	Conidia poured	4	5	171	6·8
None	Conidia poured	0	1	39	1·6

Table 2. *Infection of tomato plants in relation to root damage with scalpel cuts* in situ

Treatment	No. plants wilting	No. plants with hyphae at cotyledonary node	Mean no. vessels containing hyphae per section (means of 30 plants)
Injured, inoculated	10/30	25/30	17·1
Uninjured, inoculated	0/30	1/30	0·7
Injured, uninoculated	0/18	0/18	—
Uninjured, uninoculated	0/18	0/18	—

These results suggested that root injury facilitated invasion and that deliberate injury with a scalpel was less harmful than normal transplanting. Sections of heavily infected stems showed occlusion of the primary xylem vessels, necrosis of the adjacent xylem parenchyma cells and abnormal cell divisions in the pith parenchyma cells (Pl. 12, fig. 8).

Experiment 4. *Effect of damaging roots by cutting.* Germinated tomato seeds, cultivar Potentate, were planted in $3\frac{1}{2}$ in. pots in a sandy-loam soil mixed with granite chips and peat, to which had been added superphosphate and sulphate of potash, each at the rate of $1\frac{1}{2}$ oz. per bushel. Two levels of N were included in the experiment, 0 and 0·4 g. sulphate of ammonia per pot, but as these treatments did not greatly affect either growth of the tomatoes or number of infected plants they will not be considered further.

Ninety-six plants were raised in the glasshouse in September and, 2 weeks after sowing, random scalpel thrusts were made in the soil of half the pots. A conidial suspension (20 ml.) was poured on to the soil above thirty damaged and above thirty undamaged plants. There were thirty-six uninoculated plants, of which half were damaged by scalpel thrusts.

Two weeks after inoculation the plants were sectioned at the cotyledonary node. The results are summarized in Table 2. These two experiments have thus provided evidence that infection occurs much more readily in damaged than in undamaged roots.

DISCUSSION

These studies indicated that the degree of invasion of intact tomato roots by *V. albo-atrum* is influenced by the level of sucrose supplied in the medium. Large amounts of sucrose, such as would be supplied in an artificial nutrient medium, resulted in a high inoculum potential, leading to rapid penetration of living root cells and early establishment of hyphae in the xylem vessels. In John Innes compost, in which the fungus normally grows less profusely than in media supplied with sugar, invasion of intact roots may be restricted, at least temporarily, to the piliferous layer or the outermost layer of cortical cells. This condition was found to be paralleled in sterile culture by supplying only 200 p.p.m. sucrose. Evidently caution is necessary in applying conclusions based on sterile culture work in artificial media to the infection process in soil.

The external sucrose supply also affected lignituber formation. With 200 p.p.m. an interaction occurred between the invading hyphae and the contents of the host cell, resulting in the inclusion of the hyphae by a thick wall, but when 20,000 p.p.m. was supplied, lignitubers were small, few in number and the enclosed hyphae were observed to break through the tips of the lignitubers and continue growth.

Whether the condition of the host cells can affect lignituber formation or breakdown is uncertain. Indeed, in the present experiments, supplying sucrose to roots may have reduced their capacity to interact with the invading hyphae. If it be supposed that the host cell contributed to the wall material of the lignituber, factors leading to an increase in the internal sugar supply, such as high light intensity, or N deficiency, might be expected to limit the rate of systemic invasion. No effect of N supply on invasion was found, but in these experiments the growth of the host was not visibly affected by the level of N. In view of the important influence of N on infection of tomato by *V. albo-atrum* reported by Roberts (1943), Derbyshire (1956), and others, this merits further study. It would seem, however, that the effect of low N in reducing the incidence of infection is unlikely to be directly upon the inoculum potential of the fungus, but is probably dependent on its effect on host metabolism.

The studies made with transplanted seedlings, or with plants damaged by cutting, gave clear evidence that exposing injured roots to spore suspensions of the fungus resulted in rapid systemic invasion of the host. Other workers (Selman & Pegg, 1957; Spencer, 1957) have failed to show any appreciable increase in infection as a result of deliberate root damage but, in these cases, the 'undamaged' control plants had been transplanted. Under normal conditions of root growth in soil, where the inoculum potential of the fungus may be expected to be relatively low, it seems probable that entry of the xylem vessels occurs largely through wounds and not via living tissues. That this conclusion is at variance with some early work on the subject may be attributed to the fact that sucrose previously had been supplied in the external medium in amounts exceptionally favourable to the fungus.

The authors gratefully acknowledge the assistance of Miss R. Brett in preparing the photographs.

REFERENCES

BEWLEY, W. F. (1922). 'Sleepy disease' of the tomato. *Ann. appl. Biol.* **9**, 116–133.

DERBYSHIRE, D. (1956). The relation between mineral nutrition of the tomato plant and infection with *Verticillium albo-atrum*. Ph.D. thesis, London University.

DUFRÉNOY, J. (1927). Étude expérimentale des relations des *Verticillium* et de leur hôte. *Rev. Path. vég.* **14**, 207–214.

ISAAC, I. (1946). Verticillium wilt of sainfoin. *Ann. appl. Biol.* **33**, 28–34.

JOHANNSEN, D. A. (1940). *Plant microtechnique*. New York: McGraw Hill.

ROBERTS, F. M. (1943). Factors influencing infection of the tomato by *Verticillium albo-atrum*. *Ann. appl. Biol.* **30**, 327–331.

SELMAN, I. W. & GRANT, S. (1957). The influence of temperature and daylength on spotted wilt virus disease of tomatoes. *Ann. appl. Biol.* **45**, 312–317.

SELMAN, I. W. & PEGG, G. (1957). An analysis of the growth response of young tomato plants to infection by *Verticillium albo-atrum*. *Ann. appl. Biol.* **45**, 674–681.

SPENCER, D. M. (1957). Studies on direct and systemic fungicidal action. Ph.D. thesis, London University.

TALBOYS, P. W. (1958). Some mechanisms contributing to *Verticillium* resistance in the hop root. *Trans. Brit. mycol. Soc.* **41**, 227–241.

VAN DER MEER, J. H. H. (1925). *Verticillium* wilt of herbaceous and woody plants. *Meded. LandbHoogesch., Wageningen*, **28**, 1–82.

EXPLANATION OF PLATES 11 AND 12

PLATE 11

Fig. 1. T.S. of infected primary root of tomato grown in sterile nutrient solution with 20,000 p.p.m. sucrose. The cortex is completely invaded (× 150).

Fig. 2. Similar to Fig. 1. Note hypha growing away from the tip of a small lignituber on the wall separating a cell of the piliferous layer from an outer cortical cell (× 1350).

Fig. 3. L.S. of inoculated root of tomato grown in sand. The piliferous layer is heavily infected and many lignitubers project into the outer cortical cells. The cortex is not invaded (× 600).

Fig. 4. T.S. of root as in Fig. 3. Note nucleus of an outer cortical cell closely associated with large lignitubers (× 1800).

PLATE 12

Fig. 5. Surface view of cleared, inoculated root of tomato grown in sterile nutrient solution with 200 p.p.m. sucrose. Relatively few lignitubers are present (× 500).

Fig. 6. Similar to fig. 5 but with 2000 p.p.m. sucrose. Many lignitubers are present (× 500).

Fig. 7. T.S. of inoculated primary root of tomato grown in sterile nutrient solution with 200 p.p.m. sucrose. Note hyphae in the piliferous layer and lignituber projecting into an outer cortical cell. The cortex is not infected (× 1800).

Fig. 8. T.S. of cotyledonary node of infected tomato stem, of transplant grown in John Innes compost. The primary xylem is heavily infected and abnormal division of the adjacent pith parenchyma cells has occurred (× 500).

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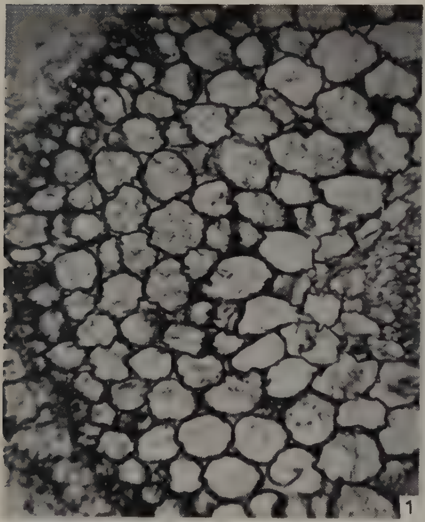

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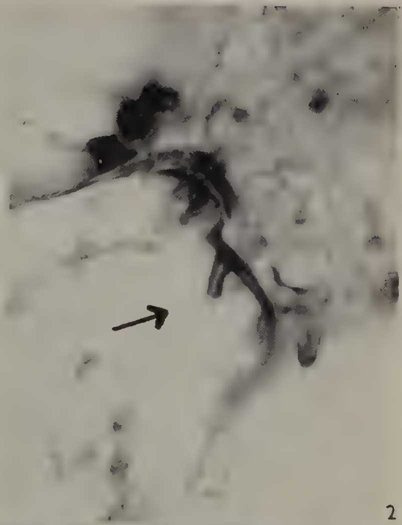

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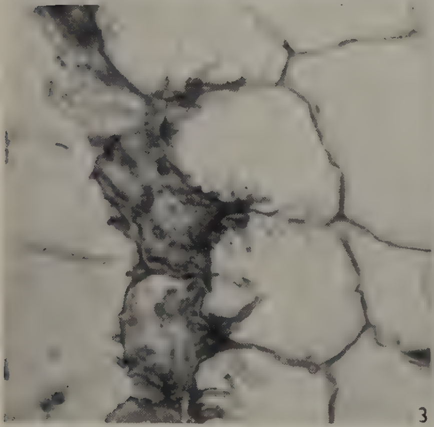

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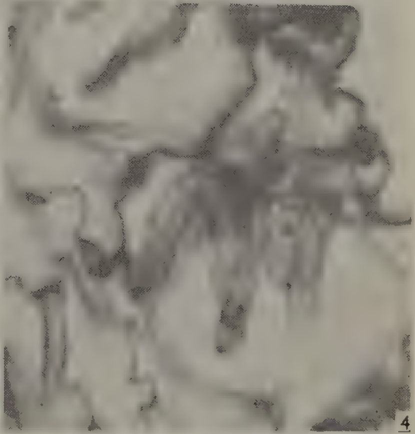

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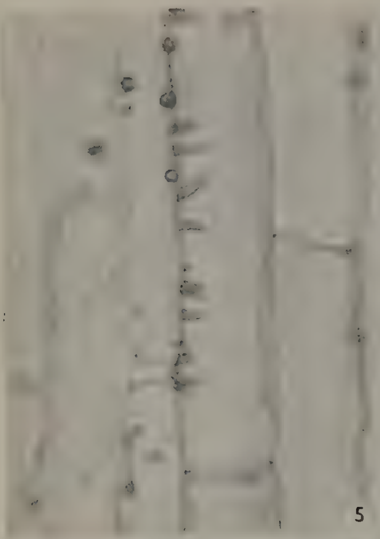

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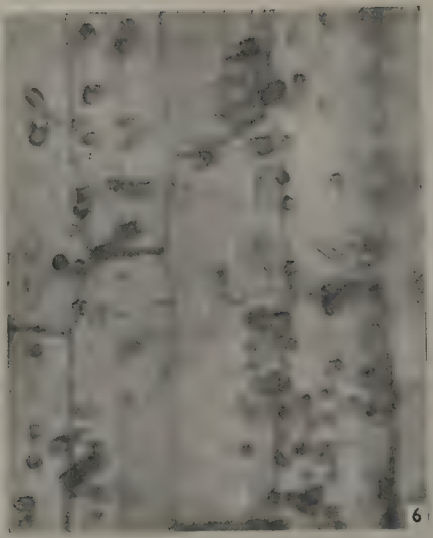

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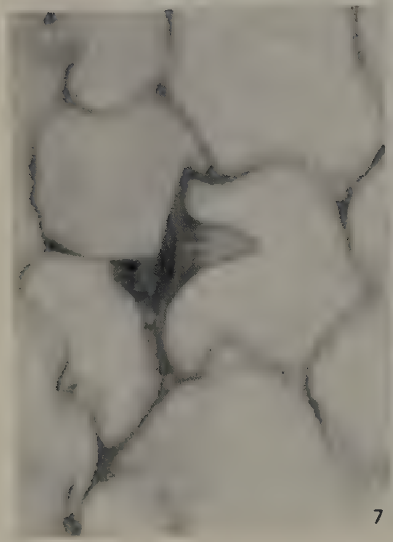

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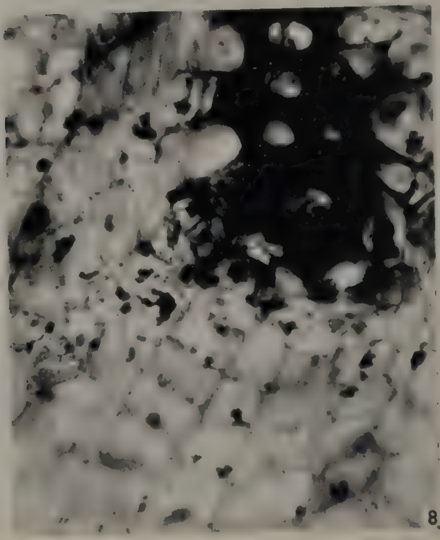

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Trans. Brit. mycol. Soc. **42** (2), 235–242 (1959).

EFFECT OF INOCULUM DILUTION ON SPORE GERMINATION AND SPORELING GROWTH IN A MUTANT STRAIN OF *ASPERGILLUS AMSTELODAMI*

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(With Plate 13 and 1 Text-figure)

Single spore cultures of a mutant strain of *Aspergillus amstelodami* grew slowly or not at all on media which supported rapid growth of heavy inocula. Retardation was partly due to slowing of the germination process and of the linear rate of sporeling growth, but much more to lack of hyphal branching.

A Cellophane technique was used to condition agar media by heavy inocula in such a way that they afterwards supported normal development of single spore cultures. Carbon dioxide, added to the incubation atmosphere, stimulated sporeling branching but did not improve germination or rate of linear growth.

In attempting to make single spore isolations of a strain of *Aspergillus amstelodami* (Mangin) Thom & Church, a physiological abnormality resulting in defective growth from sparse inocula was noted. It was found that spores seeded thinly on Sabouraud or solid Czapek-Dox media produced sporelings which were slow to branch and develop a mycelium, or sometimes failed to develop altogether. Heavier inoculations on the same media grew rapidly. The media could be 'primed' to support normal growth of single spore colonies by first germinating a heavy inoculum on a disc of Cellophane laid on the agar surface and then stripping this off, leaving the plate ready for re-inoculation with single spores, or with sporelings from already germinated suspensions. The familiar term 'staled' seems inappropriate in this connexion, hence our term 'primed' which is convenient and descriptive.

MATERIALS AND METHODS

The strain of *A. amstelodami* was maintained by mass transfers of spores on Sabouraud slants. Incubation was at 30° C. unless otherwise stated. Spore suspensions and dilutions were made in 6% glucose solution. Conidia were used in most experiments and were obtained from young Sabouraud cultures, 4–10 days old. For ascospore suspensions, cleistocarps were taken from older cultures. They were freed from conidia by manipulation on an agar surface, transferred to a sterile microscope slide, and the ascospores liberated by rupture of the sacs. Czapek-Dox agar (but with 12·5% sucrose) was most frequently used, but Sabouraud agar gave the same results.

The 'priming' technique was as follows. Discs of Cellophane (British

Cellophane Ltd., No. PT 300) were cut to size, interleaved with filter papers and autoclaved in a large Petri dish. Just before use, each Cellophane disc was steeped for 1 min. in sterile distilled water to facilitate smooth application, excess water was touched off on filter-paper and the disc laid on the agar surface in a 9 cm. Petri dish containing 15 ml. medium. The moist Cellophane was easily drawn into position so as to cover the agar surface without wrinkling or air bubbles. The plate was closed and inverted and a grease-pencil mark made on the glass to delimit a central area about 5 cm. diam. for inoculation. This area was inoculated by loop direct from a Sabouraud slant with an inoculum of spores heavy enough to be visible to the naked eye. After incubation for 1 or more days, the Cellophane with growing inoculum was stripped from the agar surface and discarded. The medium, so conditioned, was the 'primed' medium used in the experiments described below.

Comparisons were made of the development of spores from the same suspensions on primed and unprimed plates. Spore suspensions and dilutions immediately after preparation, or after a preliminary germination period of 1 or 2 days, were distributed as droplets calculated to contain approximately one spore per drop on the surfaces of the plates at ringed sites. Development was followed microscopically without opening the plates. After an interval for germination and early growth, but before much branching had occurred, each site was labelled with the number of spores it had received. A reasonable proportion had single spores, but on some occasions each site had several spores, giving a total plate inoculum of over 100 spores. Some plates were also set up with larger inocula. Spores were sown in the same way on small agar blocks cut from primed and unprimed medium. The blocks were segregated on glass slides and observed in moist chambers improvised from Petri dishes.

Where plates were incubated in carbon dioxide-enriched atmospheres, the carbon dioxide was obtained from cylinders and was admitted to a partially evacuated jar under manometric control, to give the desired mixture with air. The effects of biotin and thiamine were tested by incorporating sterile solutions in the melted agar before pouring plates.

Two typical strains of *A. amstelodami*, used for comparison, were obtained from the Commonwealth Mycological Institute (I.M.I. nos. 16110 and 57394).

RESULTS

Single spores, or sporelings from germinated suspensions, sown on primed media developed into colonies visible to the naked eye after 3 days at 28 °C., whereas controls sown on unprimed medium took at least 5 days and more commonly 7 to 8 days to reach the same stage of development, if they developed at all. On the second day, the difference under the microscope was striking. Young colonies on primed medium showed a complex surface and subsurface mycelium, branched and rebranched to a high degree and beginning to sprout aerial hyphae, while the control sporelings showed merely an elongated germ tube with little or no branching. Elongation rather than branching continued to characterize the latter throughout their early development, resulting in a very thin mycelium.

Sometimes sporelings did not branch at all but continued to elongate for several days, appearing under the microscope as single hyphal threads extending across one, two, or more low-power fields. More commonly a gradual development of hyphal branching occurred so that, at the end of a week, when the primed plates had mature, sporulating colonies, the unprimed plates had colonies with a thin mycelium just visible to the naked eye. These then went on to sporulate and eventually caught up with the others. Conidia and ascospores behaved exactly alike.

The priming effect of a heavy inoculum was quite quickly produced and remained constant, without going on to exhaustion or staling of the medium during incubation beyond the sporulation stage of growth. Thus media which had been primed by only 20 hr. growth of a very heavy inoculum on Cellophane gave normal growth of single spores, as did media primed by 4 days growth.

The retardation of single spore development on unprimed medium operated from the earliest germ tube stages and there was no difference as a result of germinating suspensions before sowing—that is, if these suspensions were dilute. If a heavy spore suspension was allowed to germinate and then used to prepare working dilutions, the sporelings were more advanced at sowing and showed more vigorous development than was otherwise the case; nevertheless, there was still a marked contrast between their progress on primed and unprimed medium.

The retardation of development ceased to operate when the amount of mycelium, however slowly it might have formed, reached a favourable proportion relative to the surrounding medium.

Both the total number of the spores inoculated on an unprimed plate and the pattern of their distribution affected growth. The total number affected general rate of development throughout the plate, whereas local inequalities in distribution affected individuals, so that clusters of more than three or four spores eventually outstripped those of one or two on the same plate.

The total number of spores on an unprimed plate, when evenly distributed, needed to be large to give enhancement of growth comparable with that resulting from priming with a really heavy inoculum on Cellophane. There was, however, no threshold; rather a continuous improvement in the rapidity and quality of early development as the inoculum was increased from less than 100 to more than 10,000 spores per plate.

Non-viable spores inoculated in large numbers along with a small number of viable spores did not assist the development of the latter. This was demonstrated by seeding an unprimed plate with a heavy inoculum of mixed conidia and ascospores from a culture nearly two years old. All the conidia and many of the ascospores were non-viable but a few of the latter (about 1 in 20) did germinate. These showed the same abnormal development as single spores planted in isolation.

On several occasions it was observed that single spores seeded on tiny blocks of unprimed agar (2 mm. cube), and incubated in a moist chamber, developed more rapidly than similarly incubated spores on larger blocks ($8 \times 5 \times 2$ mm.).

When plates were primed by growing the Cellophane inoculum over a limited area only, and the surface was then seeded thinly with spores, a gradient of development was seen outside the boundary of the primed area. Up to 5 mm. outside the margin, spores showed much the same rate of development as those within it but those farther out were increasingly retarded, and in this region the degree of retardation from spore to spore was uneven, suggesting slight variations in their growth capacities. Some spores among those seeded on unprimed media appeared to develop more rapidly than could be accounted for by variations in growth capacity and these soon proved to be contaminated by other fungi, i.e. to be benefiting as a result of 'mixed culture'. In one case the contaminant, a *Penicillium* species which was not identified, was isolated and used successfully to prime medium for the mutant strain of *A. amstelodami*.

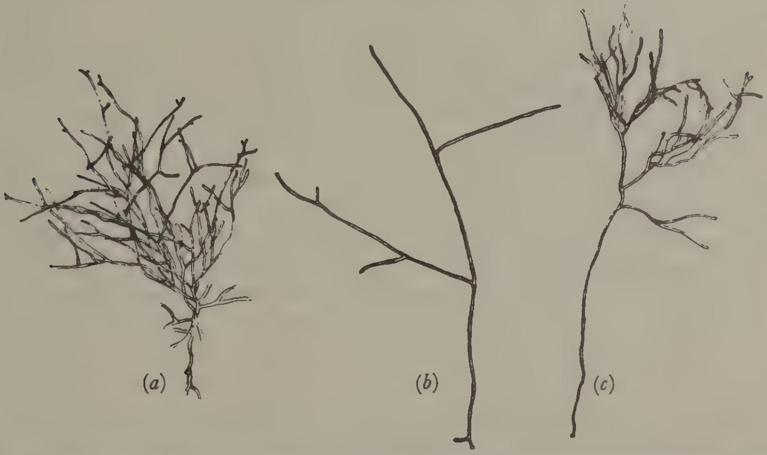
Biotin and thiamine, added to Czapek-Dox agar (10 % sucrose) at the rate of 5 μg./100 ml. medium, had no effect on the growth of the fungus from single spores. Carbon dioxide, in concentrations of 10 % and over, had a stimulating effect on the branching of sporelings growing on unprimed medium. This was not accompanied by an improvement in the rate of linear growth, which indeed was somewhat retarded, but the greater ability to branch and produce a mycelium resulted in accelerated colony development (Pl. 13, A and B). Spore germination was not markedly affected; if anything, it was slightly retarded.

With primed medium, incubation in carbon dioxide-air mixtures resulted in retardation of colony development (Pl. 13, C and D). Branching was already adequate and the slowed rate of hyphal elongation became evident as an adverse effect.

The most striking effect of carbon dioxide was seen when sporelings from a germinated suspension were sown at a fairly advanced state of development on unprimed medium and incubated for 24 hr. before adding the carbon dioxide. At this stage there was simple branching and the hyphae were elongating steadily. When incubation was continued in 10 % carbon dioxide for a further 24 hr. the sporelings had a striking appearance. From nearly every hyphal tip, a leash of branches was developing, giving a broom-like appearance (Text-fig. 1 *c*). Sporelings remaining in air without carbon dioxide showed only further elongation (Text-fig. 1 *b*). When 40 % carbon dioxide was used, elongation was inhibited altogether, but after 24 hr. the sporeling hyphae had become thick and swollen-looking each with a number of boss-like projections, representing incipient branching points. These made rapid mycelial growth when transferred to 10 % carbon dioxide (Text-fig. 1 *a*).

To eliminate the possibility that the defective development observed in this strain was due to some unsuspected toxic factor in the media rather than to an inherent physiological defect, two other strains of *A. amstelodami* (I.M.I. nos. 16110 and 57394) were studied under the same experimental conditions. These showed normal development from isolated spores, without retardation or lack of branching, on the same media and under the same conditions of incubation as those under which development of the mutant strain was defective.

There was, then, in this mutant a metabolic inadequacy seen in the isolated sporeling growing on ordinary media. This inadequacy resulted in a slow rate of linear growth and in deficient branching. It ceased to be expressed when the cumulative effect of the growing mycelium on the medium became sufficient and was not expressed at all, even in the early stages, if the sporeling had the support of a large number of companions around it, or if the diffusible products of their growth remained in the medium as a result of the Cellophane priming technique.



Text-fig. 1. *Aspergillus amstelodami* mutant; early sporeling development on unprimed medium. The three sporelings are representative of differently treated plates and are drawn side by side to the same scale for comparison. In the case of example (*a*) only one quadrant of the colony has been drawn. (*a*) Incubated 24 hr. without added CO_2, 24 hr. in 40% CO_2 and 24 hr. in 10% CO_2; complex mycelium. (*b*) Incubated 3 days without added CO_2; elongation and simple branching. (*c*) incubated 48 hr. without added CO_2 and then 24 hr. in 10% CO_2; simple, elongated hyphal pattern giving place to complex branching. Camera lucida drawings, all ×40.

DISCUSSION

To explain the defective growth of sparse inocula as described above, it is necessary to postulate some dialysable substance, produced during growth and necessary for growth, which diffuses so rapidly into the medium that isolated sporelings are unable to build up a sufficient concentration in their vicinity and so are at a disadvantage compared with heavy inocula. Such a substance must be of small molecular size to have so rapid a rate of diffusion. Its nature remains a matter for hypothesis though some possibilities have been considered and ruled out.

First an accessory growth factor requirement of the vitamin B group was considered. In favour of this hypothesis were certain resemblances to previous observations where factors of this group were involved. For instance, the adverse effect of inoculum dilution was reminiscent of that which Wildiers (1901) noted in the yeast experiments which led him to postulate the existence of 'Bios'. But it was not identical in mechanism for, with inocula consisting of spores from an aerial mycelium, there was no carry over of medium to provide the necessary factors as was the case with Wildiers's yeast inocula. Also in the present case maintenance was possible on synthetic medium alone, whereas Wildiers's yeast soon ceased to grow when serially subcultured in synthetic medium, because the growth factors, carried over initially from malt, soon became exhausted. Again, the beneficial effects of 'mixed culture' and of 'primed' medium were somewhat similar to effects which were observed in connexion with the growth and fruiting of *Melanospora destruens* Shear and which Hawker (1939) found to be explained by requirements for biotin and thiamine (Asthana & Hawker, 1936). But she did not report any abnormality of early development or inoculum dilution effect, nor any 'priming' effect of a heavy inoculum of the same strain; with *M. destruens* it was medium 'staled' by other fungi which stimulated growth and sporulation. These dissimilarities, however, between the present and the earlier observations were explicable on the basis that the requirement in the present instance was not absolute as in the others, but only partial, i.e. a limited ability to synthesize some B group factor, but in barely sufficient amount for growth, was supposed. Against this hypothesis, however, there was the important fact that the phenomenon occurred on Sabouraud medium, which one would not have expected to be deficient in factors of the vitamin B group. This also made the possibility of an amino-acid requirement seem very unlikely.

A more likely hypothesis was a requirement for one of the organic substances which have been shown to stimulate the germination of fungal spores. The simplest of these substances is carbon dioxide. Durrell (1924, 1925) showed that the addition of carbon dioxide to the incubation atmosphere resulted in an increased germination of *Basisporium gallarum* spores. A similar effect was observed by Platz, Durrell & Howe (1927), who found that 15% carbon dioxide was optimal for germination of chlamydospores of *Ustilago zeae*. But these investigators did not report any inoculum dilution effect or any abnormality of sporeling growth. In bacterial studies, however, an inoculum dilution effect due to a high requirement for carbon dioxide was detected in *Brucella abortus* (Smith, 1924); the larger the inoculum the better was growth and the less the need for additional carbon dioxide, since the organisms themselves produced a certain amount of the gas. Many other bacteria have been shown to require carbon dioxide, though this is not apparent under ordinary conditions. Walker (1932) showed that *Escherichia coli* would not grow if carbon dioxide was continually removed from the incubation atmosphere and Gladstone, Fildes & Richardson (1935) showed the same for all seven species of bacteria which they examined. They also noted an inoculum dilution effect under carbon dioxide-deprived conditions. Ajl & Werkman (1948) studied the effect of certain dicarboxylic acids (Lwoff & Monod, 1947) and other substances in replacing carbon dioxide in the metabolism of *E. coli* and *Aerobacter aerogenes*, and concluded that the effective substances were those concerned in the Krebs cycle, either directly or as precursors. The literature on the subject has been summarized in Topley and Wilson's *Principles of Bacteriology and Immunity* (1955). A carbon dioxide-requirement of the *Brucella abortus* type might have adequately explained the phenomenon we have described, and indeed the addition of 10–15% carbon dioxide to the incubation atmosphere did have a striking effect.

Where sporelings had reached a certain stage of development on unprimed medium, branching was strongly stimulated. Yet when spores were incubated in carbon dioxide-air mixtures from the start, germination and germ-tube elongation were retarded and so also was the rate of linear growth of hyphae at later stages. Except for the stimulation of branching, then, the effect was not comparable with that produced by priming the medium or by growing heavy inocula and it was concluded that the phenomenon could not be adequately explained on the basis of a simple carbon dioxide effect. Nevertheless, an explanation involving carbon dioxide in a secondary role was indicated, leading to the following hypothesis.

The mutant strain of *A. amstelodami* is postulated to have a defect in whatever primary oxidative process yields the supply of energy and basic materials needed for synthesis and growth in this species. The defect is in the production of some substance of small molecular size which either itself acts as a catalyst, or is a necessary part or precursor of a more complex catalytic system (e.g. a prosthetic group for an enzyme). The defect is not absolute and some synthesis is possible. Carbon dioxide, produced as a by-product of the oxidative process, is itself necessary for a secondary chain of synthesis responsible for branching. If this hypothesis is near the truth it is necessary to explain the adverse effects of carbon dioxide on the rate of germination and of linear growth, and it still remains to identify the catalytic substance. This we have not attempted to do.

Regarding the adverse effects of carbon dioxide which we have noted, these correspond to the retarding effects on spore germination and growth of many fungi, as observed by Brown (1922*a*). This retardation was not due to oxygen deficiency resulting from the replacement of air by carbon dioxide but to a specific effect of the latter. Brown (1922*b*) also investigated the stimulation of spore germination in *Botrytis cinerea* by various plant tissues (e.g. apple slices) placed in the same incubation vessel with the spore suspensions, and showed that carbon dioxide was not the factor involved. However, distillates of plant leaves did stimulate germination, as did also ethyl acetate and other esters of ethyl alcohol. In Brown's studies, germination was measured in terms of 'average germ-tube length' after stated periods of incubation, rather than in terms of percentage germination and this brings his studies slightly nearer the present work than most observations of a similar kind. Of these there are many reports, including those of stimulation of germination by dung infusions, extracts of plant tissues and various volatile chemical compounds (Lilly & Barnett, 1951), but as they seem to concern only the germination and not the ensuing aspects of growth their relevance to the present problem is doubtful.

One study is of considerable interest, however. Smart (1937) showed that spore germination in a number of species of Myxomycetes was increased by the use of 'previous germination medium', i.e. liquid medium in which spores of the same species had previously been germinated and which had then been filtered. Smart called the factor concerned an 'autocatalytic agent' and this term well describes the action of whatever substance is involved in growth of the strain of *A. amstelodami* described here. The mutant character of this strain and the different class of fungus involved make it unlikely, however, that there is any identity in the substances concerned.

We wish to thank Dr N. G. Heatley, Oxford, for the initial observation of the inoculum dilution effect, for suggesting the Cellophane technique, and for much interest in these studies. We also wish to thank Prof. Dick for his advice and criticism in writing this paper. One of us (W.M.D.) is indebted to the Northern Ireland Hospitals Authority for a grant during the later stages of this work.

REFERENCES

AJL, S. J. & WERKMAN, C. H. (1948). Replacement of CO_2 in heterotrophic metabolism. *Arch. Biochem.* **19**, 483.

ASTHANA, R. P. & HAWKER, L. E. (1936). The influence of certain fungi on the sporulation of *Melanospora destruens* and of some other ascomycetes. *Ann. Bot., Lond.*, **50**, 325.

BROWN, W. (1922*a*). On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and carbon dioxide. *Ann. Bot., Lond.*, **36**, 257.

BROWN, W. (1922*b*). Studies in the physiology of parasitism. IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. *Ann. Bot., Lond.*, **36**, 285.

DURRELL, L. W. (1924). Stimulation of spore germination by CO_2. *Science*, **60**, 499.

DURRELL, L. W. (1925). Basisporium dry rot of corn. *Bull. Ia agric. Exp. Sta.* **84**. (Abstr. in *Exp. Sta. Rec.* (1925), **53**.)

GLADSTONE, G. P., FILDES, P. & RICHARDSON, G. M. (1935). Carbon dioxide as an essential factor in the growth of bacteria. *Brit. J. exp. Path.* **16**, 335.

HAWKER, L. E. (1939). Accessory growth factors influencing growth and fruiting of *Melanospora destruens* Shear. *Ann. Bot., Lond.*, **3**, 657.

LILLY, V. G. & BARNETT, H. L. (1951). *Physiology of the fungi.* New York: McGraw-Hill.

LWOFF, A. & MONOD, J. (1947). Essai d'analyse du rôle de l'anhydride carbonique dans la croissance microbienne. *Ann. Inst. Pasteur.* **73**, 323.

PLATZ, G. W., DURRELL, L. W. & HOWE, M. E. (1927). Effect of carbon dioxide upon the germination of chlamydospores of *Ustilago zeae* (Beckm.) Ung. *J. agric. Res.* **34**, 137.

SMART, R. F. (1937). Influence of certain external factors on spore germination in the Myxomycetes. *Amer. J. Bot.* **24**, 145.

SMITH, T. (1924). Some cultural characters of *Brucella abortus* (Bang) with special reference to CO_2 requirements. *J. exp. Med.* **40**, 219.

TOPLEY, W. W. C. & WILSON, G. S. (1955). *Principles of bacteriology and immunity*, 4th ed. revised by Wilson, G. S. & Miles, A. A. London: Edward Arnold.

WALKER, H. H. (1932). Carbon dioxide as a factor affecting lag in bacterial growth. *Science*, **76**, 602.

WILDIERS, E. (1901). Nouvelle substance indispensable au développement de la levure. *Cellule*, **18**, 311.

EXPLANATION OF PLATE 13

Single spore colonies of *Aspergillus amstelodami* mutant under different conditions of growth (6 days' incubation). All plates were incubated at the same time with the same suspension of germinated spores.

(A) Unprimed medium; no addition of carbon dioxide during incubation.
(B) Unprimed medium; 16 % carbon dioxide during days 2–4 of incubation.
(C) Primed medium; no addition of carbon dioxide.
(D) Primed medium; 16 % carbon dioxide during days 2–4 of incubation.

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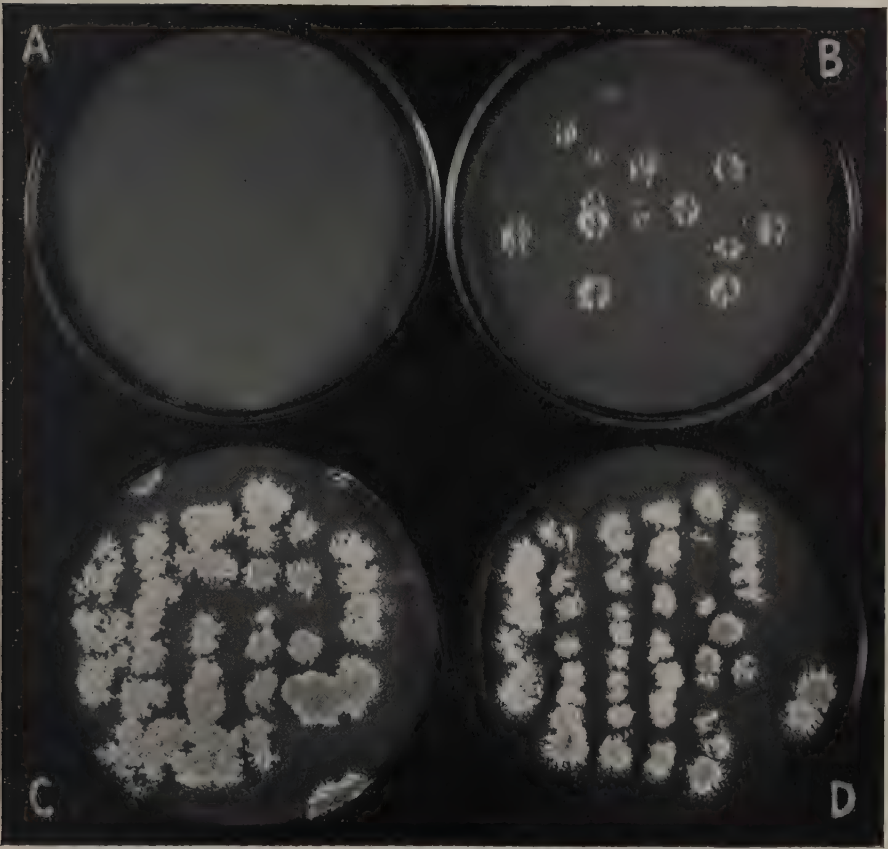

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DISPERSAL OF *FOMES ANNOSUS* FR. AND *PENIOPHORA GIGANTEA* (FR.) MASSEE

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(With 1 Text-figure)

Measurements of spore deposition in forests, obtained by exposing freshly cut sections of pine stem, indicate that in relatively calm conditions rates exceed 20 spores/100 cm.^{2}/hr. close to sporophores. Deposition rates of 1–5 spores/hr. are commonly encountered in well-ventilated plantations where local spore sources occur. A simple method is described of trapping spores on muslin squares: they are later recovered by shaking with sterile water and plating the suspension on pine sections. By this means *Fomes annosus* and *Peniophora gigantea* were found to be regular components of the air spora in Britain. At selected places the rate of deposition on muslin squares was determined in winds of varied direction, and for both species rates of over 20 spores/100 cm.^{2}/hr. were recorded close to forests. Rates declined with distance from source, but in the Shetland Islands, some 200 miles from source, the two fungi and other forest species were still detectable. Another method is given for determining the number of *F. annosus* spores on samples of conifer foliage. Examination of samples from some British forests shows that spores are widespread and vary greatly in abundance; there is some relation with proximity to sporophores. The limitations of this method for assessing the risk of stump infection in plantations are discussed.

INTRODUCTION

In the work described here an attempt was made to discover how frequently spores of some wood-inhabiting fungi occur in the air. Special attention was paid to *Fomes annosus* Fr. and *Peniophora gigantea* (Fr.) Massee, an important competitor of the former in conifer stumps. Rates of deposition under various conditions have been studied and some indication obtained of the distances over which viable spores are carried. Freshly cut, unsterilized sections of Scots pine stem (Rishbeth & Meredith, 1957) were utilized as a medium throughout. This complex substratum, comprising dead and living tissues and exudates from living cells, is highly selective for *F. annosus*, *P. gigantea* and, to a lesser extent, some blue-stain fungi. When sections are autoclaved this selectivity is destroyed and common moulds grow as freely as on malt agar, for instance. Under most conditions wood-inhabiting fungi are not readily detected in the air by agar media owing to their suppression by moulds, although considerable selectivity is claimed for a medium devised by Russell (1956). Methods involving microscopic examination of spore deposits are hardly applicable since spores of the fungi in question are small and commonly lack distinctive morphological features (although *F. annosus* spores are somewhat unusual in containing oil droplets). Estimates of viable spores are in any case to be preferred since their proportion to the total number produced varies considerably; for *F. annosus* this proportion is commonly 1–5 % and for *P. gigantea* over 50 %. These two species are perhaps especially convenient for dispersal studies. They are mainly to be found in conifer plantations so that in regions of varied land utilization spore sources are widely scattered. In Britain, spore production is almost continuous throughout the year, and the large area of some spore sources facilitates detection at considerable distances. Where large areas of forest are in early stages of thinning, information about dispersal of *F. annosus* may help to assess the risk of stump infection by the pathogen and the liability of plantations to subsequent attacks of killing or butt-rot.

Exposure of wood sections

The simplest and most direct method of studying spore deposition of *F. annosus* in forest is to expose freshly cut pine sections horizontally on the ground. Relatively smooth-barked lengths of pine stem 10–12 cm. in diameter, cut not more than 10 days previously, are lightly scraped to remove loose bark scales, but without cutting living bark, and are then swabbed thoroughly with 95 % alcohol. A saw blade is similarly swabbed and sections about 1 cm. thick sawn off. Mechanical sawing appears to be unsatisfactory, probably because of surface heating. Sections are wrapped in sterile newspaper and then moistened: before and after exposure moisture loss is restricted by storage in polythene bags. For exposure the section is placed on the newspaper, folded inwards, for periods ranging from 10 to 90 min. Periods of 30–45 min. commonly give satisfactory results, but it is often desirable to make exposures of varied duration where deposition rates are unknown. Longer exposures, especially in open situations, often cause marked drying, which appears to affect selectivity for *F. annosus* adversely. This difficulty can be partially overcome by exposing sections in a shallow container, resting on wet cotton-wool and held in position by rubber sheeting. Sections, together with an unexposed control, are incubated at 22° C. for 10–15 days in a container allowing some access of air. After 10 days the surface is examined for the characteristic conidia of *F. annosus* with a × 10 hand-lens, the position of colonies being marked by wax pencil. In addition, it is helpful to view the section as a semi-transparent object in front of a strong light. *F. annosus* colonies are often seen as lenticular shadows, but later they may become wedge-shaped. *F. annosus* slightly darkens the wood surface whereas *P. gigantea*, which commonly appears a day or two later, stains it reddish brown. *P. gigantea* colonies often spread rapidly and are sometimes radially elongated but more often are irregular in outline; after 12–14 days the characteristic chains of oidia are usually visible. *F. annosus* is overgrown fairly rapidly by *P. gigantea* during incubation and is thus difficult to detect where the latter is abundant; to a lesser extent the reverse is also true. In East Anglia, *Ceratocystis pilifera* (Fr.) Moreau is the next most common species to form conspicuous colonies. The many species producing inconspicuous colonies have been merely noted; *Cladosporium herbarum* Fr. and *Penicillium* spp. are among the commonest. *Trichoderma viride* Pers. ex Fr., and to a lesser extent *Botrytis cinerea* Pers. ex Fr., tend to grow vigorously if the wood is not fresh or the bark inadequately cleaned: in such instances basidiomycetes are often suppressed. When final counts have been made the diameter of the wood surface is measured and results are expressed as the number of viable spores deposited/hr./100 cm.^{2}, an average area for first-thinning conifer stumps. Sections with 5–20 colonies are the most satisfactory for this purpose.

This method has been used mainly in Thetford Chase, East Anglia, in pine plantations 20–35 years old, where *F. annosus* is locally very abundant. Table 1 shows the distribution in various categories of deposition rates for *F. annosus* and *P. gigantea* spores, as calculated from exposures made over the period March 1957–August 1958.

Table 1. *Frequency distribution of deposition rates for* Fomes annosus *and* Peniophora gigantea *spores on exposed pine sections*

(No. samples (out of 85) falling into categories tabulated hereunder)

	Deposition rate (viable spores/100 cm.^{2}/hr.)					
	Not detected (< 1)	1–5	6–20	21–60	61–150	> 150
F. annosus	28	24	15	10	6	2
P. gigantea	16	27	27	12	2	1

Table 2. *Data on spore deposition in five plantations*

	No. exposures			Mean deposition rate (viable spores 100 cm.^{2}/hr.)	
		No. developing			
Site	Total	*F. annosus*	*P. gigantea*	*F. annosus*	*P. gigantea*
A	14	12	11	4	8
B	8	6	4	14	5
C	14	13	8	80	6
D	7	2	5	0·6	10
E	10	3	8	0·5	23

About half the exposures give *F. annosus* depositions in the range 1–20 spores/100 cm.^{2}/hr. and some two-thirds show *P. gigantea* spore deposition of the same order. Table 2 summarizes results obtained from five plantations over a period of about a year.

Sporophore abundance and distribution play a major role in determining frequency of spore deposition at any particular site. *F. annosus* sporophores are almost confined to stumps and to infected trees and vary greatly in abundance; thus at site A they were common, at sites B and C very common and at sites D and E uncommon. By contrast, sporophores of *P. gigantea* are found not only on stumps but on fallen branches and dead suppressed trees and are more evenly distributed throughout the area; rates of spore deposition correspondingly vary rather less. Considerable variation may be found even within a plantation. Exposures were made in experimental plots 1 chain wide which had received different stump treatments 10 years previously. On three plots where stump infection by *F. annosus* had largely been prevented by painting or creosoting, *F. annosus* was not detected (twice) or was deposited at the low rate of

3 spores/100 cm.^{2}/hr. (once). On three adjacent plots where stumps had not been protected and *F. annosus* sporophores were frequent, deposition rates were 12, 17 and 18 respectively.

At a given site, deposition in the vicinity of sporophores tends to be greatest when the air is relatively still. The freedom with which air moves near ground-level in a plantation correspondingly affects the average rate of spore deposition there. Thus in Table 2, site E is not as well ventilated as D, and sites A–C form a series of decreasing ventilation, as determined by conditions ranging from a high tree canopy with no undergrowth and a relatively open situation to a low canopy with thick undergrowth and a sheltered situation. At site C the average rate of spore deposition for *F. annosus* is exceptionally high and here, too, the highest rate so far recorded, 280 spores/100 cm.^{2}/hr., occurred in mid-November 1957. By contrast, the deposition rate for *P. gigantea* was often lower at this site than in the immediate vicinity, probably because spore drift, originating mainly from outside, was impeded by the thick vegetation. Simultaneous exposure of horizontally and vertically disposed sections indicates that in very calm conditions spore deposition occurs predominantly as sedimentation (Gregory, 1952). In very light winds rates of deposition on vertical and horizontal sections may be similar, and in moderate winds a few spores may be deposited on the lower surface of sections exposed some 50 cm. above ground-level, demonstrating the occurrence of turbulent deposition. The part played by the rough wood surface of a section or stump in spore deposition is problematical, but in light winds as many *P. gigantea* spores are deposited on well-planed sections as on rough ones.

Effect of weather on spore deposition

Changes in weather affect spore deposition of *P. gigantea* markedly, but that of *F. annosus* to a lesser extent. After only a few dry days in spring or summer, *P. gigantea* seldom appears on sections exposed at situations where deposition is heavy during showery weather, whereas *F. annosus* deposition is hardly affected. In the plantations, where spore sources are generally close, heavy spore deposition of both species has been found to occur directly after prolonged rain. Laboratory experiments show that sporophores of *F. annosus* are more sensitive to freezing than those of *P. gigantea.* Only one out of five sporophores of the former species deposited spores at room temperature after storage for 20 hr. at -3° C., whereas two sets of three sporophores of the latter produced copious deposits of viable spores after a similar period of storage at -3° and -12° C., respectively. Under East Anglian conditions, measurements of spore deposition during winter give little indication of this, however. Although water-soaked *F. annosus* sporophores may be found in more open situations after heavy frost, the majority are sufficiently sheltered to continue discharging. After a fortnight of intensely cold weather in March 1958, moderate deposition rates (6–15 spores/100 cm.^{2}/hr.) were recorded at several sites when the air temperature was only 1° C. By contrast, deposition rates of *P. gigantea* are often low after periods of cold weather, and observations suggest that this is due to drying out of the sporophores rather than to freezing. It seems likely that with intensely cold winters, as in northern Europe, spore production by *F. annosus* is reduced to a very low level.

Apart from such temporary effects of weather, there is no evidence from section exposures of any definite periodicity in *P. gigantea* spore discharge throughout the year. However, in East Anglia *F. annosus* spore discharge appears to decline during mid- to late-summer. At site C, *F. annosus* deposition was not observed to decline below 40 spores/100 cm.^{2}/hr. over the period November 1957–April 1958 but did not exceed 5 during July and August 1958. At site B this latter period was the only one during the year in which rate of *P. gigantea* spore deposition exceeded that of *F. annosus*. Some blue-stain fungi show marked periodicity: thus in two successive years *Ceratocystis piceae* (Münch) Bakshi appeared abundantly only on sections exposed towards the end of April. Deposition of *C. pilifera* occurred much more regularly, often at rates of 1–10 spores/100 cm.^{2}/hr.

Similar spore deposition rates for *F. annosus*, *P. gigantea* and *C. pilifera* were recorded on a recent visit to Sweden, where many of the stands visited were rather older than in Thetford Chase and were known to be attacked by *F. annosus*. At many sites *F. annosus* sporophores were to be found only under stumps of butt-rotted spruce slightly lifted by the wind, but spore deposition occurred at rates comparable with those at sites having exposed sporophores. Spore drift from hidden sporophores could be detected by placing sections beneath outlying roots of undisturbed stumps. Thus in well-ventilated situations, air currents near ground-level are sufficient to disperse spores of *F. annosus*, and presumably other fungi, from hidden sporophores, the importance of which as spore sources has sometimes been doubted.

Since *P. gigantea* is one of the most important competitors of *F. annosus* in freshly cut stumps, a series of section exposures at a given place could indicate the pattern of stump infection likely to occur. This is borne out at some of the sites listed in Table 2; a series of plots thinned at monthly intervals at site E had a high incidence of stump infection by *P. gigantea* almost throughout, whilst several instances of heavy *F. annosus* stump infection have occurred in the vicinity of sites B and C. However, stump infection seldom follows a consistent pattern in a given situation, largely owing to short-term variations in the relative abundance of *F. annosus* and *P. gigantea* spores (Rishbeth, 1957). From the practical viewpoint, exposures made at the time of thinning or felling might well provide information about the probable incidence of stump infection, but this would be too late to affect any decision about the desirability of stump treatment, which must be immediate. And although the method enables spore deposition to be measured in forests, where sources are relatively abundant, it is less suited for remote sites where rates of deposition are low, as for example in many young plantations on moorland.

Exposure of muslin squares

For studying dispersal of forest fungi over greater distances, the need arose for a simple method of collecting samples of air spora on a non-perishable substratum from which spores could subsequently be recovered. In the method eventually adopted (Rishbeth, 1958) pieces of muslin 20 cm. square are folded and placed in transparent 'self-sealing' envelopes, which are then dry-sterilized at 100° C. and stored until required. For exposure, a muslin square is withdrawn from an envelope and attached to a wire frame about 18 cm. square, previously swabbed with 95 % alcohol, by means of a small paper clip ('Bulldog' type) at each corner. The frame is fixed in a suitable holder, such as a bamboo stick, so that the muslin faces squarely into the wind, supported behind by the frame's cross-wires. Static exposures are made in any open situation about 2 m. above ground; exposures may also be made from a moving vehicle. The muslin is exposed for a period ranging from a few minutes to several hours, according to conditions, and is then folded and returned to the envelope, which is resealed. The catch is estimated by cutting a 10 cm. square from the centre of the muslin, transferring it to a screw-cap bottle and shaking with 20 ml. sterile water for 2 min. After compressing the material to expel excess water, the suspension and suitable tenfold dilutions are plated with malt agar to estimate mould spores; in addition, 5 ml. suspension is transferred to a freshly cut pine section 10–12 cm. diam. and 2 ml. to each of two sections of about 6 cm. diam. If desired, 2 ml. of the dilutions may also be transferred to pairs of smaller sections. Large sections are incubated in moist newspaper and small ones in Petri dishes, under the conditions already described. Sections of both sizes receive an equivalent volume of sterile water and are then incubated as controls. Subsequent examination is as before. The chief advantage of this method is that exposures may be made at a number of places simultaneously; the envelopes (each weighing about 3 g.) and frame can readily be sent by post. By virtue of the dilution method, wide variations in rate of spore deposition present no serious problem, by contrast with section exposures.

To enable the method to give quantitative results, possible sources of variation and error were investigated. Under moderately uniform conditions in an open situation, during the afternoon, size of catch of mould and basidiomycete spores was found to be proportional to time of exposure over the range 15–240 min. It is thus possible to express rate of catch as viable spores/100 cm.^{2} muslin/hr. With much longer periods of exposure, a mean rate determined in this way would often conceal much variation. Wind speed would be expected to influence rate of catch. When exposures were made in a wind-tunnel of cross-section 21 × 28 in., rate of catch of mould spores was proportional to air speed over the range 5–30 m.p.h. At 5 m.p.h. the commonest species trapped was *Trichoderma viride*, whereas at 20 m.p.h. *Cladosporium herbarum* predominated. This may well be connected with the tendency of the first species to form larger spore groups, some of them over 100 μ in diameter, which implies high efficiency of impaction at low air speeds (Gregory, 1952). Small variations in wind direction probably do not affect rate of catch greatly; in fact, rate of catch is high if a muslin is exposed vertically, but parallel with the wind direction. However, if muslin is exposed horizontally instead of vertically in a moderate wind, the rate of catch is smaller. Simultaneous exposures in the same situation generally give closely comparable results.

With respect to recovery, variations in count between samples from the same muslin are no greater than would be expected from use of the dilution method. Nevertheless, it is possible that errors arise through variation in the proportion of spores removed from different muslins. Serious errors could arise through death of spores before estimation; in general, the aim was to limit the interval between exposure and estimation to a week at the most. It is sometimes necessary to repeat an estimation and various methods of keeping exposed muslins were tested, the most successful being storage in the dark over anhydrous calcium chloride. By this means counts of *F. annosus* and *P. gigantea* very similar to the original could be obtained after 2 months or more. The sensitivity of the method in detecting such species in a sample of air spora is considered later. Clearly, estimates of spore deposition obtained by this method are subject to many variables and there is the additional disadvantage that such estimates cannot be related to the volume of air sampled or to the total air spora. However, the simplicity of the method allows considerable scope for operation, and a study of spore deposition on a standard surface under natural conditions is not without interest.

By this method, *F. annosus* and *P. gigantea* spores have been detected at many places in Britain. Of 140 static exposures made from July 1957 to August 1958, 83 (59 %) yielded the former species and 114 (81 %) the latter. The extent to which detection depends on the selectivity of pine sections is shown by the fact that the two species appeared only 3 and 4 times, respectively, on malt agar plates prepared from muslin suspensions. Other wood-rotting basidiomycetes seldom produce easily recognizable colonies on pine sections but sometimes appear on malt agar plates when moulds are infrequent. *Stereum sanguinelentum* (A. & S.) Fr. was the species most commonly encountered in this way. Three other species characteristic of conifers appeared occasionally: *Sclerophoma pithyophila* (Corda) Hoehnel could be identified on malt plates, whereas *Alternaria abietis* Tengwall and *Ceratocystis pilifera* formed easily recognizable colonies on pine sections.

Exposures were made at several places to investigate the effect of wind direction during predominantly dry weather. Cambridge was a convenient centre at which to make regular exposures and was not especially close to any major source of *F. annosus* spores. Thetford (Norfolk) and Orford (Suffolk) were chosen on account of their proximity to known spore sources of *F. annosus* and *P. gigantea*; Port Erin (Isle of Man), Den Helder (Holland) and Scalloway (Shetland Islands) for their remoteness from them. The potentialities of Exeter (Devon) and Caernarvon (North Wales) in this respect were unknown. Exposure periods ranged from a few hours to 12 hr. or more at places remote from forests; wind velocities were most commonly in the range 3–6 of the Beaufort scale. Table 3 gives some of the results which indicate only roughly the rates of catch to be expected at such places.

Cambridge lies in an area mainly of arable, and there are many scattered woodlands, predominantly of hardwoods but often with an admixture of conifers. This situation is perhaps reflected in the tendency for *P. gigantea* spores to drift from all directions. There are scattered small sources of *F. annosus* in the vicinity but major sources in Thetford Chase and adjacent forests lie 25–50 miles away in the direction N.N.E.–E.N.E. A more detailed analysis of results than can be given in Table 3 shows that of 12 exposures made in winds coming from this direction, 11 yielded *F. annosus*, the average rate of catch being 4 spores/100 cm.^{2}/hr. Of 18 exposures made in other wind directions, 8 yielded *F. annosus* and the average rate of catch was only 1 spore/100 cm.^{2}/hr. There was no corresponding difference between the two series in frequency or rate of catch of *P. gigantea*.

Table 3. *Effect of wind direction on rates of catch of* Fomes annosus *and* Peniophora gigantea *spores on muslin squares*

	Approximate wind direction							
	N.	N.E.	E.	S.E.	S.	S.W.	W.	N.W.
	Viable spores/100 cm.^{2}/hr.*							
Cambridge	—	8, 5	0, 3	0, 5	4, 15	0, 4	1, 17	1, 5
Thetford	—	1, 1	1, 1	1, 0	—	14, 64	30, 11	23, 2
Orford	1, 19	1, 3	0, 1	—	0, 6	0, 1	3, 2	5, 50
Exeter	—	0, 1	0, 1	0, 0	1, 2	3, 12	2, 8	1, 2
Caernarvon	—	0, 4	1, 3	—	—	—	4, 6	0, 0
Port Erin	0·3, 1	—	—	0·3, 1	—	—	0·5, 1	0, 0
Den Helder	0, 0	—	1, 1	0, 1	—	—	0, 0	0, 0
Scalloway	—	0, 0·3	0, 0	0, 0	1, 0·3	—	0, 0	—

* The first figure of each pair represents the catch of *F. annosus* spores and the second that of *P. gigantea* spores.

Thetford lies just to the east of the older and extensively thinned portions of Thetford Chase. Exposures were made at Gallows Hill, an open site overlooking plantations in which areas heavily infected with *F. annosus* are to be found within 1–2 miles in an arc extending from south-west to north. Exposures in these directions trapped unusually large numbers of *F. annosus* spores, even when allowance is given for the strong winds in which they were made. *P. gigantea* spore counts were sometimes very high, but not consistently so. By contrast, catches of the two species from younger plantations to the east were uniformly low. *Alternaria abietis* and *Ceratocystis pilifera* were also trapped at this site.

Orford lies near the east coast. From the point of exposure at Gedgrave, the nearest major sources of *F. annosus* lie some 3–4 miles to the N.W. in Tunstall forest. Some 4–6 miles to the west, Rendlesham forest has several badly affected plantations, but nowhere in the district is *F. annosus* so widespread as near Thetford. With respect to wind direction, *F. annosus* counts conform more or less to expectation and are considerably lower than at Thetford. One *P. gigantea* count, for an exposure in wind coming from the nearest heavily thinned area, is very high however.

Exeter is interesting because of the unexpected drift of *F. annosus* and *P. gigantea* spores from south-west to west. It was discovered that Haldon forest, lying 5–7 miles from the point of exposure in this direction, was extensively planted with conifers and that parts had received several thinnings. *Stereum sanguinolentum* and *Alternaria abietis* were detected only in winds from this quarter.

At Caernarvon exposures similarly disclosed the presence of a considerable spore source in Anglesey, not as yet identified.

Port Erin had no potential sources nearer than the mainland in directions ranging from south-east through west to north. Exposures recorded spore drift of *F. annosus* and *P. gigantea* from the general directions of N. Ireland (50 miles), Galloway (50 miles) and Lancashire (80 miles). The appearance of exposed muslins roughly indicates the extent of air pollution with smoke, and it was a useful check on wind direction that of the muslins exposed here only one, in a south-east wind (Lancashire), was discoloured. A local spore source was almost certainly detected by one exposure made in a very light wind, variable in direction, *P. gigantea* being trapped at a rate of 9 spores/100 cm.^{2}/hr.

Den Helder, on the coast of Holland, is similarly far removed from large forest areas. From the east, small numbers of *F. annosus* and *P. gigantea* spores were detected and must have drifted some 50 miles over recently reclaimed land and open water. No basidiomycetes were recorded in a long westerly exposure, but *Alternaria abietis* was detected and may well have been carried over 120 miles from East Anglia.

Scalloway, Shetland Islands, probably lies 180 miles from the nearest major sources of *F. annosus* in north and east Scotland. At this distance, conclusions based on wind direction at the point of exposure could be very misleading; however, considerable care was taken here to select appropriate conditions. The only catch of *F. annosus* recorded was from the general direction of east Scotland, known to contain extensive spore sources. *P. gigantea*, *A. abietis* and *C. pilifera* were trapped in winds from the direction of Norway, about 250 miles away.

Although it is possible to determine the origin of spores by this method in some circumstances, it is usually difficult because of inability to discriminate between a close, relatively weak source and a distant, stronger one. For this reason, too, it is often impossible to determine how far *F. annosus* spores drift over land from a major source. The East Anglian fens, being virtually devoid of conifer plantations, provide an excellent opportunity for studying such drift. Preliminary observations indicated that in an east wind *F. annosus* spores could be detected for at least 15 miles along a line running roughly north to south, some 10 miles from Thetford Chase. Later, simultaneous exposures were made in an E.S.E. wind of average velocity 12 m.p.h. at various distances from the forest. Rates of catch (spores/100 cm.^{2}/hr.) for *P. gigantea* were 60 at 2 miles, 2 at 10 miles and 0·4 at 20 miles. *F. annosus*, which on this occasion was not as abundant, was detected at 2 and 10 miles, but only with difficulty, owing to the prevalence of *Trichoderma viride*. The latter species was not detected further than 15 miles from the forest, however. Of the characteristic forest fungi, only *Alternaria abietis* was obtained at 30 miles.

For rapidity of detection, exposure from a vehicle travelling between 20 and 40 m.p.h. was often convenient. Thus a static exposure in a plantation near Thetford trapped 3 *F. annosus* and 15 *P. gigantea* spores/100 cm.^{2}/hr., whereas a simultaneous 'mobile' exposure in the immediate vicinity trapped 6 *F. annosus* and 45 *P. gigantea* spores/100 cm.^{2}/hr. The greater abundance of *F. annosus* spores near the source is well illustrated by results of such exposures; for 7 exposures made in forest areas the average rate of catch was 4/100 cm.^{2}/min., whereas for 8 exposures made well outside the rate was 0·6. The various localities in Britain at which *F. annosus* spores have been detected by muslin exposures are recorded in Fig. 1. Of 16 exposures made on ships in the North and Irish seas, 5 yielded *F. annonsus* and 4 yielded *P. gigantea*. The highest rate of catch for *F. annosus* was one of 8 spores/100 cm.^{2}/min. obtained just off the Norwegian coast at Målöy (62° N.) and the fungus was also detected near Trondheim (63° 30′ N.) by this means. Another successful exposure was made in a west wind 30–40 miles from the coast of Eire, when the rate of catch for both *F. annosus* and *P. gigantea* spores was 0·1/100 cm.^{2}/min.

Relatively few exposures have been made in Europe, but in Sweden *F. annosus* was detected in the counties of Värmland and Östergötland and in Holland at Leeuwarden and Den Helder. At the extreme south of its European range, *F. annosus* was detected at Malta by a long exposure in a north-east wind; *P. gigantea* and *A. abietis* were also trapped. The nearest possible spore sources were pine plantations in Sicily some 150 miles distant, from which, however, *F. annosus* has not been recorded.

Rates of catch for mould spores have not been analysed in detail, but a similar decrease in numbers with distance from source is shown by the average rates of 40–50 spores/100 cm.^{2}/hr. obtained for exposures at Port Erin and Scalloway, as compared with one of about 1000/hr. for exposures at Orford over a similar period. At inland stations, rates of catch for moulds often lie in the range 200–600 during winter and in the range 1000–3000 during summer. Spores of *F. annosus* and *P. gigantea*, as estimated by plating on pine sections, together often comprise some 1–2 % of the number of mould spores estimated on agar plates, but the proportion tends to rise near their source, as might be expected, and in plantations may exceed 20 %. The proportion of the two species in the air spora tends to be lower in summer, since there is apparently much less seasonal variation in numbers than with moulds.

As expected, wind velocity commonly affected rate of catch. Thus in simultaneous exposures, rate of catch of *F. annosus* and *P. gigantea* was twice as great in an open ride with freely moving air as it was under tree canopy a few yards away. In almost still air it was sometimes possible to demonstrate the existence of a concentrated spore cloud, as with section exposures, and in one instance *F. annosus* spores were deposited at a rate of 70/100 cm.^{2}/hr. In general, however, the low air speeds prevalent under tree canopy result in rates of catch of basidiomycetes similar to those obtained several miles from forest, where higher wind velocities compensate for dilution of the spore cloud. An exceptional rate of catch was obtained in an open situation, in very light wind and continuous drizzle, when 70 *P. gigantea* and 30,000 *Sporobolomyces* spores/100 cm.^{2}/hr. were trapped. Gregory & Sreeramulu (1958) note that such conditions favour high concentrations of hyaline basidiospores. The effect of snow cover in curtailing spore production was observed at several sites. Thus at Gallows Hill, Thetford, in January 1958, rates of catch for *F. annosus*, *P. gigantea* and moulds were 14, 64 and 1000 spores/100 cm.^{2}/hr. respectively; a fortnight later, after snowfall, exposures in the same wind direction yielded no *F. annosus* or *P. gigantea* and only 50 mould spores/100 cm.^{2}/hr.

To assess the significance of these observations for stump infection, it is important to know the relation between the rates of deposition on muslin and on horizontally disposed sections at ground level, under comparable conditions. The first comparisons were made in forest in light to very light winds, and under these conditions agreement was good. However, when comparisons were made in moderate to strong winds, agreement was poor, rates of deposition on muslins often being five times greater than those on sections.

SURFACE MICROFLORA OF CONIFER NEEDLES

The discovery that pine needles bear large numbers of fungal spores, including basidiospores, and that the proportions of species on them vary with the locality (Rishbeth & Meredith, 1957) raises the question as to how far counts of *F. annosus* spores on needles could be used as an index of the spore potential of a given site. The mere presence of *F. annosus* spores shows that stump infection by the pathogen is a possibility that must be considered, but it would be convenient, especially with plantations as yet lacking spore sources, if some indication of the degree of risk could be obtained. For sites already having stump infection of varying extents, information about spore production derived from needle counts could be a useful supplement to survey work in the plantations.

In the method of estimation chosen, a foliage sample is collected by breaking off shoot tips at intervals along the edge of a forest 'ride' and at about 6 ft. above ground. From this, 40 g. of short shoots (about 500) for pines, or 5 cm. lengths of 'extension' shoot (about 50) for other species, is weighed and transferred to a sterile 1 l. flask. Sterile water (100 ml.) is added and a surface-sterilized rubber bung substituted for the cotton-wool plug. After shaking the flask for 2 min., the suspension and one or two tenfold dilutions are plated out on to two sizes of pine section, as for muslin samples. Incubation and examination are also similar. Counts are calculated as viable spores per 10 g. foliage (fresh weight) and are hereafter quoted in this way. Samples are estimated as soon as possible after collection and are stored in bags at 4° C., in case repeated estimation is necessary.

As with the previous method, attempts were made to determine sources of variation and error. Because of the great variety of fungal species likely to be present on foliage, it was thought desirable to check the sensitivity of the method in detecting *F. annosus* spores. To a suspension prepared from a sample known to carry little *F. annosus*, varying numbers of basidiospores, as calculated by haemocytometer count, were added before estimation by the standard method. On the assumption that 3 % of the spores were viable, it was planned that three series of sections should receive 40, 4 and 1 spores respectively per 2 ml. suspension; a fourth would receive none, as control. Foliage and spore suspensions were plated separately with malt agar by the dilution method for counts of moulds and viable spores respectively. Counts on wood sections agreed well with expectation, even the smallest inoculum being detected. A similar result was obtained with *P. gigantea* spores. A parallel experiment was carried out with a suspension prepared from an exposed muslin. Selectivity was not so rigorously tested here, one *F. annosus* spore being detected in a suspension containing about 600 mould spores, mainly *Cladosporium* and *Penicillium* spp., as compared with one in some 20,000 spores, mainly *Sclerophoma* and *Cladosporium* spp., in the foliage suspension. The method is almost certainly less sensitive than this when *Trichoderma viride* or other rapid growers form a considerable proportion of the leaf spora.

For a reliable method of sampling, it is important to know something about the distribution of spores on conifer foliage. Samples were taken, as usual, from the edge of a 'ride' and from the crown of a tree growing a short distance away inside the plantation: this was done at three sites, each having a different conifer species. In all instances basidiospores were more abundant on the lateral branches ('ride' sample) than on the crown. With Norway spruce, for instance, counts were 300 and 30, respectively, for *F. annosus* and 800 and 100 for *P. gigantea*. It seems probable that the greater exposure of tree crowns to direct sunlight is mainly responsible for this effect. For purposes of detection, therefore, the more convenient 'ride' sample is also more sensitive. Along the side of a plantation there is considerable variation in spore population from branch to branch, but less between different portions of a single branch; the standard method of collection is thus preferable to single-branch samples. For counts over 100, the error for a single determination is about ± 50 %; for lower counts it is somewhat greater.

Further important points to be determined for a given site are the variation of spore counts with tree species and age. Earlier observations were confined to Scots pine, which is so widely planted as to be the most convenient species for comparisons. Especially in the north and west of Britain, however, needle samples are often obtainable only from spruces; in theory, too, it should be possible to use foliage of a ubiquitous tree such as birch. Samples were collected from two groups of trees, each composed of a variety of species, along a ride in Thetford Chase. In the first group, *F. annosus* spore counts were as follows: ponderosa pine, 1500; Douglas fir, 3000; beech, 3000; birch, 6000. In the second group they were: Norway spruce, 700; Scots pine, 700; birch, 6000. Substantial differences may thus be found between different species growing under comparable conditions. In the situation just described, uneven incidence of water-splash from higher branches might invalidate strict comparison between species with respect to trapping spores from the air. Foliage samples from young isolated trees at three open sites in Thetford Chase, taken from positions at which there was little possibility of water-splash, suggest that the tendency for broad-leaved species to carry more spores, shown above, is even more pronounced in such situations. In one instance *F. annosus* counts were: Scots pine, 10; birch, 120; sycamore, 300. A rough calculation showed that variations between species with respect to the fresh weight/surface area ratio of leaves were insufficient to account for these differences. Since leaves of many broad-leaved species are more freely moved by wind than conifer needles, turbulent deposition on them may well be greater. Young naturally regenerated trees under partial canopy often carry large numbers of spores, whereas trees of similar size in unshaded plantations up to 10 years old often have relatively few, even in areas of high spore potential. With the latter, counts are roughly comparable with those on tree crowns in older plantations, so that degree of exposure to sunlight is probably the major determining factor.

However carefully sampling were standardized, the method could still give very misleading information if spore counts varied greatly throughout the year. *F. annosus* counts for repeated samplings at a given site generally agree well, but those of *P. gigantea* often vary considerably. Clearly it is desirable to discover how fast spores on foliage accumulate and disappear. Observations on the rate of accumulation were made on pines close to a spore source of *F. annosus*. Terminal shoots of branches unlikely to be subject to water-splash were washed by vigorous shaking in a polythene bag with water and detergent, and then with water alone. Needle samples were taken before washing, directly after, and then at intervals of a few days. The *F. annosus* spore count was reduced by washing from 130 to 10, rose to 30 after 3 days and to 140 after 10 days. The *P. gigantea* count rose to the original level of 60 after only 3 days. Under such conditions, therefore, spores accumulate fairly rapidly on foliage. It seems likely that determination of the rate of disappearance of spore populations is only reliable when made at some distance from major spore sources. At the edge of a relatively isolated plantation near Cambridge, terminal shoots on lateral branches were inoculated by spraying on basidiospore suspensions of *F. annosus* or *P. gigantea*. Of two pairs inoculated with each species, one was left exposed and the other loosely covered with polythene sheeting and secured in such a position that it was protected from rain, though open below. Needle samples were taken before inoculation, immediately after, and then at intervals until spore counts did not differ appreciably from uninoculated controls. In Exp. 1, carried out in November 1957 (Table 4), several nights of heavy frost occurred before the first sampling and the weather was dry throughout.

Table 4. *Survival of* Fomes annosus *and* Peniophora gigantea *spores on pine foliage*

	Interval (days) between inoculation and sampling			
	0	15	29	43
	No. spores/g. foliage			
F. annosus				
Foliage exposed	1800	5	3	3
Foliage covered	—	90	30	6
P. gigantea				
Foliage exposed	500	2	1	—
Foliage covered	—	1	1	—

The marked reductions in spore count after 2 weeks were at that time attributed to frost, and it was shown that spore viability in the two species is adversely affected by alternate freezing and thawing. Even more rapid killing of spores occurred over the same period on small pines, with naturally established leaf-surface populations, which had been transferred to an exposed site remote from spore sources. However, similar rates of reduction in spore count were found during relatively mild weather in Exp. 2, carried out in May 1958 (Table 5).

P. gigantea spores died rapidly, only a minute proportion surviving more than 2 weeks, and there was little difference between exposed and covered foliage in this respect. *F. annosus* spores survived appreciably longer only in the covered series, a very few spores being detectable after 4 months. Heavy rain fell at intervals throughout this experiment, but further work would be required to establish whether longer survival of spores on sheltered foliage is due to protection from rain or from direct sunlight, for example. That desiccation is not a serious hazard is suggested by the continuing survival of *F. annosus* spores stored in dry soil in the dark for 16 months.

Table 5. *Survival of* Fomes annosus *and* Peniophora gigantea *spores on pine foliage*

	Interval (days) between inoculation and sampling					
	0	2	10	17	25	46
	No. spores/g. foliage					
F. annosus						
Foliage exposed	400	60	3	3	—	—
Foliage covered	—	40	50	20	10	3
P. gigantea						
Foliage exposed	700	70	2	1	—	—
Foliage covered	—	60	8	2	—	—

It seems probable that some leaf surface spores are washed off by rain, since several species have been detected in water dropping from the tree canopy just after rain (Rishbeth & Meredith, 1957). Foliage spore counts have not as yet demonstrated any marked reduction after rain, however; in fact unusually high counts of *F. annosus* and other species have sometimes been recorded from lower branches directly after heavy showers, perhaps as a result of washing downwards. As Gregory (1952) suggests, wind is unlikely to remove many leaf surface spores. Detachment of a very few *F. annosus* spores was demonstrated by exposing sections after vigorously shaking conifer foliage indoors, but attempts to achieve this by shaking tree crowns 10–20 ft. above ground in still air have, perhaps not surprisingly, failed. Such shaking causes a prompt deposition of *Trichoderma viride*, probably attached to relatively large particles.

These observations and experiments suggest that under East Anglian conditions the rate of accumulation of spores on foliage is often roughly equivalent to the rate of disappearance, although wider fluctuations can be expected at a distance from major spore sources. For evaluating risk of spore infection in a given area, determination of the number of spores on a random sample of foliage is clearly of little value. More reliable information is obtained if standard samples are taken from (i) the same species of conifer, (ii) plantations more than 10 years old, and (iii) branches at intervals along a 'ride'. It is probably best to avoid very cold weather since spore production and perhaps spore viability may be adversely affected. Even so the method cannot be expected to give results of any great accuracy, and spore counts might well be grouped in five categories, as in Table 6, where the distribution of counts is given for foliage of spruces and pines in some British forests. The appearance of *F. annosus* colonies on larger sections inoculated with 5 ml. suspension is characteristic for the various categories, which is convenient when rough estimates of needle populations are made in the field by a simplified version of this method.

Table 6. *Frequency distribution of* Fomes annosus *spore counts on foliage samples from some British conifer plantations*

	Category				
	Undetectable (0)	Very low (1)	Low (2)	Medium (3)	High (4)
Appearance of *F. annosus* colonies on larger sections	None	A few	Several, scattered	Many, often merging	± Covering surface
Calculated no. *F. annosus* spores/10 g. foliage	< 5	5–25	26–100	101–500	> 500
No. samples falling in categories	1	9	11	12	8

Samples were collected and received for examination during 1957–58, mainly during the course of a survey undertaken by pathologists of the Forestry Commission Research Branch. In this survey priority was given to forests known to have some attack by *F. annosus* and it is thus not surprising that about half the samples gave spore counts in the two highest categories. Spruce samples, constituting about one-third of the total, had a distribution of counts similar to that of pine samples. The highest recorded count for *F. annosus* was 8000, obtained from spruce in Hallyburton Forest, Perthshire, where sporophores were unusually abundant. From seven forests, samples were received both from a plantation adjacent to a source of *F. annosus* spores and from one some distance away. In five instances the *F. annosus* count for the former was greater (in three much greater); in one it was similar and in one somewhat smaller. In Kerry Forest, Montgomery, counts for *F. annosus* were 300 on spruce foliage adjacent to a spore source, 30 at a distance of 200 yd. through forest, and 6 at a distance of 1 mile.

A rather different situation existed at Bardney Forest, Lincolnshire, where the average age of plantations from which foliage samples were received was only 15 years and the average count for *F. annosus* was only 10. This is probably typical of young forest at some distance from major spore sources. By contrast, isolated shelterbelts or small plantations may have relatively high *F. annosus* counts: thus spruce at Aultnamain, Ross and Cromarty, had a count of 500 although it was at least 4 miles from the nearest source. It seems probable that trees in young forests do not accumulate many spores, partly because of the screening effect of dense foliage. An extreme instance of this was the failure to detect *F. annosus* on a large pine at Chippenham, Cambridgeshire, not more than 5 miles from a major source but surrounded by a dense screen of hardwood trees.

Dense foliage may well have the opposite effect in close proximity to sporophores, restricted ventilation leading to unusually large spore deposits on foliage in much the same way as reported earlier for section exposures. Some very high counts, especially for spruce, are probably to be explained in this way.

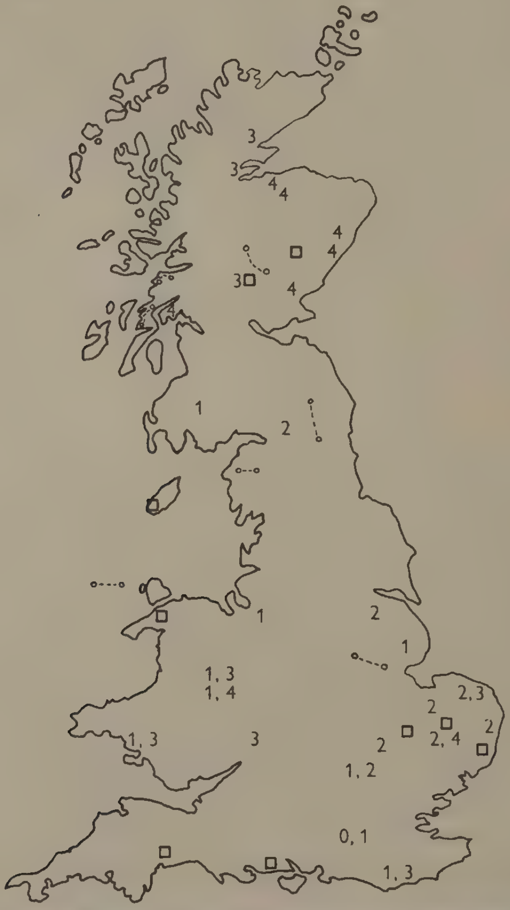

Fig. 1. Localities in Great Britain at which *F. annosus* spores have been detected on conifer foliage, or by exposing muslin squares. 0–5, categories for spore counts on foliage (Table 6); □, static muslin exposure; ○ – – – ○, mobile muslin exposure.

Categories of *F. annosus* counts for representative foliage samples from British forests are given on the map (Fig. 1). The consistently high count in east Scotland, which has a long history of conifer cultivation, is noteworthy. From these admittedly few observations, it seems likely that there are large areas of modern forest with spore counts in categories 1 and 2, with smaller pockets having counts in categories 3 and 4. Frequency of *F. annosus* spores on foliage tends to agree roughly with proximity to spore source and it seems likely that with extensive plantations, counts of 100 or more indicate the existence of a spore source within a few hundred yards. However, counts decline fairly rapidly with distance from source, so that a foliage sample can only be expected to give information about conditions in the immediate vicinity. It is probable that only where a number of scattered samples from a forest have uniformly low spore populations may it be claimed that the infection risk is as yet relatively low.

Discussion

By the methods described, it has been possible to obtain some idea of the pattern of dispersal of *Fomes annosus* and *Peniophora gigantea* spores. Although the sporophores are commonly found near ground-level, or even in cavities, and visible deposits nearby indicate great wastage, vast numbers of spores are borne away by air currents. In relatively still air, and especially in dense vegetation, deposition is often heavy in the immediate vicinity of sporophores and frequently exceeds 20 spores/hr. on a wood surface of 100 cm.^2 With better ventilation the spore cloud is soon dispersed and, in plantations 20 years old or more, deposition rates of 1–5 spores/hr. may be expected if there are local spore sources. The proportion of *F. annosus* to *P. gigantea* spores deposited, a vital factor for stump infection by the former species, depends not only on the relative abundance of sporophores but on weather; after only short dry periods, for instance, spore production by *P. gigantea* is greatly reduced. Thick screens of vegetation may appreciably restrict spore drift through forest although, as Gregory (1952) points out, fungi with such minute spores are probably to be regarded as specialized penetrators. There is little difficulty in explaining escape of spores from plantations in relatively still air: with wind blowing freely over tree crowns, many light particles can be seen drifting steeply upwards towards the canopy. At a distance of a few hundred yards from source, spores of the two fungi may be detected at a height of 80 ft. in moderate wind.

In open country and over sea, the number of viable spores falls off with distance from source, as might be expected, but some sources are so extensive that spores may be detected 200 miles away. Over shorter distances the deposition rate on a wood surface may be measured directly: in one observation, for instance, one *F. annosus* spore was deposited on 100 cm.^2 in 1 hr., 25 miles from source. At much greater distances the method of trapping does not allow precise estimates to be made, but it seems likely that, in open situations 100 miles or more from source, *F. annosus* and *P. gigantea* spores may be deposited in some conditions once every 5–10 hr. The extent to which spores from distant sources re-enter plantations to infect stumps is difficult to determine. It is interesting to speculate whether accumulation of spores on foliage increases the chance of stump infection in remote plantations; as yet there is no conclusive evidence on the point.

These observations on spore deposition are an essential preliminary to a better understanding of the occurrence of stump infection by *F. annosus* in British forests. Its spores are such a common component of the air spora and can drift so far from major sources that no area can be regarded as entirely safe from stump infection. Observations on spore deposition show that the risk of stump infection is greatly increased once *F. annosus* sporophores develop nearby. At the same time, since spore populations of potential competitors are likely to increase with the provision of stumps and cut branches, the pathogen will be controlled naturally to a varying extent. For first rotation crops, the recent history of Thetford Chase, in particular, shows that *F. annosus* may build up considerably within 10 years. For older forests, even where earlier crops were healthy, the situation can rapidly change, since thinning is now more frequent and the infection risk almost certainly greater.

The selectivity of freshly cut pine sections for *F. annosus* and *P. gigantea* was essential for detection of these species, which are amongst the most important primary colonizers of pine stumps. There seems no reason why the method should not be employed to detect primary colonizers of other tree species. For instance, by inoculating wood sections of different conifers with basidiospores of various wood-rotting fungi, it was found that although pine was the best for detecting *F. annosus*, Norway spruce was probably better for detecting *Polystictus abietinus* (Dicks.) Fr., a primary colonizer of this species. No attempt has been made in this work to determine the basis of selectivity of these complex substrata.

Grateful acknowledgement is made to Dr D. S. Meredith for help in developing the method for counting spores on conifer foliage, and to Mr B. A. Golding for much assistance in field work. The writer is also indebted to the staffs of the Research Branch and Eastern Conservancy of the Forestry Commission for collecting samples; to the directors of the Marine Biological Stations at Port Erin (University of Liverpool) and Den Helder, Holland, for facilities for collection of samples; to Prof. W. A. Mair, University of Cambridge, for permission to use a wind-tunnel, and to many others for assistance in the investigation.

REFERENCES

Gregory, P. H. (1952). Fungus spores. *Trans. Brit. mycol. Soc.* **35**, 1–18.

Gregory, P. H. & Sreeramulu, T. (1958). Air spora of an estuary. *Trans. Brit. mycol. Soc.* **41**, 145–156.

Rishbeth, J. (1957). Some further observations on *Fomes annosus* Fr. *Forestry*, **30**, 69–89.

Rishbeth, J. (1958). Detection of viable air-borne spores in air. *Nature, Lond.*, **181**, 1549.

Rishbeth, J. & Meredith, D. S. (1957). Surface microflora of pine needles. *Nature, Lond.*, **179**, 682–683.

Russell, P. (1956). A selective medium for the isolation of basidiomycetes. *Nature, Lond.*, **177**, 1038–1039.

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Trans. Brit. mycol. Soc. **42** (2), 261–269 (1959).

A STUDY OF *ERWINIA* ISOLATES OBTAINED FROM SOFT ROTS AND BLACKLEG OF POTATOES

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On cultural characteristics, but not pathogenicity, pectolytic *Erwinia* isolates from soft rot and blackleg of potatoes were divisible into three groups. When compared with named cultures and the characteristics described for the species, 16, 114, and 4 isolates were considered to represent *E. carotovora*, *E. atroseptica* and *E. aroideae*, respectively. Besides differences in response to temperature and forms of nitrogen in the medium, the action of the species on ethyl alcohol, maltose and glycerol was investigated with particular reference to the effect of the basal medium employed. It was concluded that tests for growth on special compounds such as those mentioned should be made with an inorganic basal medium and not nutrient broth or peptone, metabolic products from which could mask the reaction being investigated. Some serological tests were also made.

Bacillus atrosepticus and *Bs. phytophthorus* were described as the cause of blackleg in potatoes by van Hall (1902) and Appel (1902), respectively; a soft rot of carrots was ascribed to infection by *Bs. carotovorus* by Jones (1901) and a similar rot of calla lily, due to *Bs. aroideae*, was described by Townsend (1904). The identity and nomenclature of these bacteria have been considered by numerous authors, and at present two generic names, *Erwinia* (Winslow *et al.* 1920) and *Pectobacterium* (Waldee, 1945) may be applied. The ability to produce pectolytic enzymes serves to distinguish the soft rot group within *Erwinia*, or to separate *Pectobacterium* from it. Such enzymes are produced by other plant pathogenic bacteria, e.g. *Pseudomonas* spp. (Oxford, 1944; Paton, 1958), and for this reason it is preferred to retain the soft rot group in *Erwinia* in this paper.

Among the bacteria which cause soft rot and blackleg of potatoes, some authors have recognized three species, *Erwinia carotovora* (Jones) Holland, *E. atroseptica* (van Hall) Jennison and *E. aroideae* (Towns.) Holland, but others have regarded the three as strains of *E. carotovora*. Rudd Jones (1950) considered the findings of earlier work and, in the light of his own investigation, concluded that only one species, *Bacterium carotovorum* (Jones) Lehmann & Neumann (= *E. carotovora*) should be recognized. In similar investigations Burkholder & Smith (1949) distinguished two species, *E. carotovora* and *E. atroseptica*. In *E. carotovora* they included an isolate (E.8.A) which Dowson had regarded as *E. aroideae*, but subsequently Holdeman & Burkholder (1956) recognized *E. aroideae* as a distinct species. Hellmers & Dowson (1953) considered *Bacterium carotovorum*, *B. atrosepticum* and *B. aroideae* to be strains of *B. carotovorum*, but Dowson (1957), following Waldee (1945), has since referred them all to *Pectobacterium carotovorum* (Jones) Waldee.

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Burkholder & Smith (1949) considered *E. atroseptica* alone to be the cause of blackleg, but Hellmers & Dowson (1953) found that the disease could be induced by all three strains of *B. carotovorum*. Among the isolates tested by the latter were strains of Burkholder and Smith's *E. atroseptica* and *E. carotovora*.

In this work 134 isolates of *Erwinia* were obtained from soft rot and blackleg of potatoes, and tests were made to determine their identity and to compare them with named *Erwinia* species. The results of these tests, and a consideration of earlier studies, are presented.

MATERIALS AND METHODS

Named Erwinia *cultures*

Named cultures of *E. carotovora*, *E. atroseptica* and *E. aroideae* were obtained for comparison with previous work. *E. carotovora* was received as cultures 312, 309 and 31 from Dr W. J. Dowson, Cambridge, and cultures 8093 and 8097 from the National Collection of Industrial Bacteria, Teddington. *E. atroseptica* was received as cultures E24, 17a1 and an unnumbered isolate from blackleg in potatoes from Dr Mary Noble, Edinburgh. *E. aroideae* was received as culture 66 from Dr W. J. Dowson. It is of interest to note that 8097 was the strain used by Elrod (1941), and E24 was used by Burkholder & Smith (1949).

Isolation and selection of cultures

Isolations from blackleg and soft rot lesions on potatoes were made on meat-infusion yeast-extract agar (Dowson, 1957), which was also used to maintain the stock cultures. On this medium the *Erwinia* type of colony could be readily recognized, and it had the advantage of distinguishing the green-fluorescent bacteria which were commonly present. MacConkey agar (Ministry of Health, 1939), and modifications of it containing salicin or maltose in place of the lactose, were also used for isolation; a modification of this medium, using salicin as carbohydrate, has been used successfully by Noble & Graham (1956), but in the present studies the meat-infusion medium was preferred.

Cultures prepared from the *Erwinia* type of colony were tested to determine their ability to produce pectolytic enzymes. The bacteria were grown at 27° C. in potato-yeast extract (200 g. potato and 0·5 g. yeast extract/l. of tap water, adjusted to pH 7·2), distributed in 20 ml. quantities in 250 ml. medical flats, laid on their sides for maximum aeration. After 3 days 1 ml. of toluene was added to suppress bacterial growth, and the presence of pectolytic enzymes was determined by the disk method of Brown (1915) after 24 hr. The bacteria which produced such enzymes were selected for further testing.

Pathogenicity tests

The bacteria were tested for pathogenicity to potato tubers and stems.

In tubers a cavity, formed by removing a plug of tissue 1 cm. deep from a surface-sterilized tuber, was filled with a 24 hr. broth culture of the bacterium. The tuber was kept moist at 27° C. for 3 days, then the presence or absence of rotting was determined. All the isolates were found to cause rotting, but some were more active than others.

Pathogenicity to stems was tested on potato plants 10 in. high, using 24 hr. cultures of the bacteria on nutrient agar. The stems were pricked at the base of a petiole with a needle contaminated with the bacterium; a sterile needle was used for the controls. When the inoculated plants (two per culture) were kept at 19–27° C. a dark-coloured rot developed at the point of inoculation and usually the stems collapsed and died within a week. With a few of the cultures disintegration of the parenchyma was extensive in the medulla but only slight in the cortex round the point of inoculation. Plants affected in this way were chlorotic and stunted but did not collapse. In inoculated plants maintained at 10° C. no decay of the cortex developed but disintegration of the medulla occurred. In these plants a brown discoloration, typical of blackleg, was evident throughout the vascular strand associated with the inoculation point. All the control plants remained normal with no trace of vascular discoloration.

It was concluded that all the isolates were pathogenic to potato stems, but that the evidence of pathogenicity differed according to the post-inoculation temperature. Differences in the type of infection occurring at the higher temperature of incubation were not related to any particular group of isolates.

IDENTITY OF THE CULTURES

General characteristics

All the bacteria were Gram-negative, non-sporing rods with blunt ends. They were motile with 1–5 peritrichous flagella (Gray's staining method). Growth after 24 hr. on nutrient agar was greyish and iridescent; nutrient gelatin was liquefied, liquefaction beginning in 2 days. Salicin and lactose (1%) incorporated in a synthetic medium (0·1% $(NH_4)H_2PO_4$, 0·02% KCl and 0·02% $MgSO_4.7H_2O$; Ayers, Rupp & Johnson, 1919) with brom cresol purple as indicator, were utilized with the production of acid or acid and gas within 1 week. Glycerol (1%) in the same basal medium was utilized, with the production of acid within 1 week. Nitrate was reduced to nitrite when contained in the synthetic medium with 1% glucose, or in peptone water.

Specific characteristics

Erwinia atroseptica. One hundred and fourteen of the cultures, on comparison with the named cultures, were assigned to *E. atroseptica*. These could utilize maltose in the synthetic medium, turning the brom cresol purple yellow. The maximum temperature for growth of most isolates of this species was 32° C., a few showed some growth at 35° C., but none grew at 37° C. The thermal death point was 48° C. Among the named cultures, *E. carotovora* 309 and 31 had the characteristics of *E. atroseptica* and were consequently renamed.

Erwinia carotovora. Sixteen of the isolates were considered to be *E. carotovora*. They differed from *E. atroseptica* in being unable to utilize maltose, but able to utilize ethyl alcohol in the synthetic medium. A marked turbidity and sufficient acid to turn brom thymol blue yellow developed in the synthetic medium without added carbohydrate. In the synthetic medium without a C supplement, when the N source ($NH_4H_2PO_4$) was replaced by KNO_3 or $NaNO_2$, the nitrate and nitrite were reduced and the medium became turbid. The maximum temperature for the growth of *E. carotovora* was higher than that of *E. atroseptica*; all the cultures grew well at 37° C. and a few showed some growth at 38° C. The thermal death point was 52–55° C.

Erwinia aroideae. Four isolates differed from *E. carotovora* in having the ability to utilize maltose, but otherwise they were similar to it. These isolates and *E. carotovora* 8093 were identical with *E. aroideae* 66 and were considered to represent *E. aroideae* (= *E. carotovora* var. *aroideae* as recognized by Dowson, 1957).

CARBOHYDRATE UTILIZATION STUDIES CONSIDERED IN RELATION TO THE BASAL MEDIA USED

Ethyl alcohol, glycerol and maltose have been used previously to differentiate the pectolytic *Erwinia* species. A study of the literature has shown that in using these carbohydrates, different investigators have obtained results frequently at variance, and that different basal media or indicators have been employed. Therefore, a comparison was made of the effect of different basal media and indicators in tests for utilization of these carbohydrates. The results are presented according to the media employed.

Media containing ethyl alcohol

The use of ethyl alcohol in distinguishing between *E. carotovora* and *E. atroseptica* was introduced by Smith (1920) and has been used in several investigations. Massey (1924) used 5% ethyl alcohol in peptone agar, and distinguished *Bacillus carotovorus* (= *E. carotovora*) from *Bs. aroideae* (= E. *aroideae*), *Bs. phytophthorus* (= *E. atroseptica*) and *Bs. atrosepticus* (= *E. atroseptica*) by its production of acid, an indication of alcohol utilization.

Burkholder & Smith (1949) used 5% ethyl alcohol in 5% beef-extract peptone broth. *E. carotovora* caused the medium to become alkaline, then finally acid, while with *E. atroseptica* it became alkaline only. These results have been confirmed by Noble & Marshall (1952), but they used 1% peptone broth in place of the basal broth used by Burkholder & Smith.

In the present studies the medium recommended by Noble & Marshall, and an inorganic basal medium (Ayers *et al.* 1919), both with the addition of 5% ethyl alcohol, were used to test the named cultures and the fresh isolates. In the latter medium, cultures assigned to *E. carotovora* and *E. aroideae* grew and produced sufficient acid to turn brom cresol purple yellow in 16 days at 27° C.; with *E. atroseptica* the medium did not become turbid or acid. The medium containing peptone became acid and remained so with *E. aroideae* and most strains of *E. carotovora*; with *E. atroseptica* and the four strains of *E. carotovora* which utilized alcohol in the synthetic medium, the medium became acid in 24 hr., but became alkaline within 3 days and remained so. *E. atroseptica* and the four aberrant strains of *E. carotovora* gave identical results when grown in 1 % peptone as a basal medium without alcohol. In previous carbohydrate fermentation studies with these species, using peptone, pH reversal has been recorded (Wolf & Foster, 1921; Lacey, 1926). The action of the bacteria on the peptone alone may have masked their ability to utilize ethyl alcohol; therefore, the results indicate that in tests for the utilization of ethyl alcohol, the alcohol should be incorporated in an inorganic basal medium.

Carbon-free basal medium

In the course of the carbohydrate utilization studies, a turbidity developed and acid was produced by *E. carotovora* and *E. aroideae*, but not by *E. atroseptica*, in the 'control' tubes of the inorganic basal medium with no C supplement. By using chemically clean glassware and omitting the indicator (a possible C source), *E. carotovora* and *E. aroideae* were found to be deriving their C from some source external to the culture tube. The inoculated medium became turbid and acid (sufficient to turn brom thymol blue yellow) within 7 days when the cultures were incubated among actively fermenting cultures at 27° C.; this did not occur consistently in cultures kept for 28 days in the normal atmosphere of the laboratory, and was absent in cultures from which carbon dioxide was excluded.

The exact C source which allowed growth of these bacteria was not determined, and *E. carotovora* and *E. aroideae* are not considered to be autotrophic. The observations indicate that in critical tests for carbohydrate utilization by these bacteria, care must be taken to avoid recording growth and acid production resulting from utilization of an extraneous carbon source.

Media containing glycerol

Culture media containing glycerol have been used by several authors in differentiating among the pectolytic *Erwinia* species. When peptone has been used as a basal medium (Waldee, 1945; Burkholder & Smith, 1949), production of acid by *E. carotovora* has served to distinguish it from *E. atroseptica*. However, acid production has been recorded for both species when they were grown in a synthetic medium plus glycerol (Burkholder & Smith, 1949; Rudd Jones, 1950).

The isolates used in the present study all produced acid in the synthetic medium plus glycerol. When peptone was used as a base, acid was not produced by *E. atroseptica* and the strains of *E. carotovora* which gave the same reaction as *E. atroseptica* in the peptone-alcohol medium. In this case, as in the ethyl alcohol tests, the action of the bacteria on peptone again appeared to mask their action on glycerol. Therefore, the utilization of glycerol is not a satisfactory criterion for differentiating the species.

Media containing maltose

E. atroseptica has generally been credited with the ability to utilize maltose, but with *E. carotovora* contradictory results have been obtained. Dowson (1940, 1941) separated *Bacterium carotovorum* (= *E. carotovora*) from *B. phytophthorum* (= *E. atroseptica*) and *B. aroideae* (= *E. aroideae*) because of its inability to ferment maltose in a synthetic medium with brom cresol purple as indicator. In this medium Burkholder & Smith (1949) recorded growth of, but no colour change by, *E. carotovora*, but sufficient acid was produced by *E. atroseptica* to turn the medium yellow. They did not regard the action on maltose to be a reliable means of separating the species since they differed only in the amount of acid they produced. Rudd Jones (1950) also used maltose in the synthetic medium, but with brom thymol blue as indicator. He obtained variable results with his strains of *E. carotovora* so he did not consider maltose to be a satisfactory differential substance.

Maltose in organic basal media has been used by Lacey (1926), Elrod (1941) and Waldee (1945). Elrod used maltose in nutrient broth with brom cresol purple, and his organisms formed two groups, one of which was distinguished by its inability to produce acid or gas in the medium. Lacey and Waldee used maltose in peptone; the former author distinguished *Bacillus phytophthorus* (= *E. atroseptica*) from the other soft rotting strains because it produced acid and gas, while the latter concluded that none of the soft rot bacteria fermented maltose. Lacey used litmus and Waldee brom cresol purple. Since brom cresol purple indicates acid at a much lower pH than litmus, if insufficient acid was produced to turn the brom cresol purple yellow, the different indicators used could account for the different results obtained by these two investigators.

Maltose incorporated in nutrient broth (Difco) with brom cresol purple, maltose in peptone with brom cresol purple, and maltose in the synthetic medium with brom cresol purple or brom thymol blue were used to test the bacteria employed in the present investigation. These media without the maltose supplement were used as inoculated 'controls', and tubes of uninoculated media were used for comparison.

With maltose in nutrient broth and in the synthetic medium, *E. atroseptica* produced a moderate turbidity and sufficient acid to turn the indicator yellow, but when the maltose was incorporated in a 1 % peptone base the medium became turbid and no acid was produced. In the 'control' media growth and acid were recorded in the nutrient broth, growth but no pH change in the peptone, and no growth or pH change in the synthetic medium.

With *E. carotovora* a turbidity and change in pH occurred in all the organic media containing maltose, and the pH was lowered sufficiently to change the brom thymol blue, but not the brom cresol purple, to yellow in the synthetic medium. Identical results were obtained in the 'control' media. When inoculated tubes of the synthetic medium, with and without maltose, were incubated in air-tight jars to prevent contamination with extraneous C, no growth or change in pH was observed; in this series the indicator was added at the end of the experimental period.

E. aroideae behaved similarly to *E. atroseptica* in the media containing maltose, except that it produced acid when the peptone base was used. In the absence of maltose a turbidity developed but no acid was recorded in the synthetic medium with brom cresol purple; otherwise the maltose-free basal media became turbid and sufficiently acid for the indicator to become yellow, but the change in pH was registered more slowly than when maltose was present.

As a result of these tests neither the nutrient broth nor the peptone are considered to be satisfactory basal media, because the action of the bacteria on them alone is similar to that occurring in the presence of maltose; therefore their use could give rise to misleading results.

From the tests with *E. carotovora* in the synthetic medium it was evident, again, that utilization of extraneous C could be registered in error as utilization of a given C source.

It was concluded that *E. atroseptica* and *E. aroideae* could utilize maltose with the production of acid, but that *E. carotovora* could not.

SEROLOGICAL STUDIES

The possibility of distinguishing the coliform soft rot bacteria by serological means has been considered by Lacey (1926) and Elrod (1941). In order to determine whether serology could aid identification of the bacteria isolated in the present investigation, antisera for *E carotovora* 312, *E. atroseptica* E24, and *E. aroideae* 66 were prepared.

In cross-agglutination tests the serum prepared against *E. atroseptica* agglutinated all the *E. atroseptica* isolates tested, and the heterologous titres (60,000) were equal to the homologous titre in every case. Cross-agglutination occurred with the *E. atroseptica* isolates and the *E. aroideae* antiserum, but in this case the heterologous titres were not constant (4000–16,000), and they were much lower than the homologous titre (60,000). No agglutination occurred between the *E. carotovora* isolates and the serum prepared against *E. atroseptica*. The few isolates of *E. aroideae* tested were agglutinated by the *E. aroideae* antiserum only and, except in one case where the titre was equal to the homologous titre, the heterologous titres (16,000) were less than the homologous titre. None of the strains were agglutinated with the *E. carotovora* antiserum.

In somatic agglutination tests, agglutination took place with twelve strains of *E. carotovora* and *E. aroideae* antiserum. The titre was equal to the homologous titre (16,000) with eight of the strains; with the remainder it was 8000. Using the *E. atroseptica* antiserum, the homologous and heterologous titres were greater than 16,000, except with two strains where titres of 4000 and 8000 were obtained.

Before any definite conclusions can be drawn, further investigations with a wider range of antisera are required, using more isolates and taking into account the reactions to both the flagellar and somatic components. However, the results so far indicate a relation between maltose fermentation and the serological groups, and are in agreement with the results of earlier investigators (Lacey, 1926; Elrod, 1941).

DISCUSSION

Differences in pathogenicity among coliform soft-rot bacteria have on occasion aided in separating the species (Lacey, 1926; Dowson, 1941; Burkholder & Smith, 1949), while similarities in their pathogenicity have been presented as evidence that they should be regarded as one (Harding

& Morse, 1909; Stapp, 1928; Leach, 1930; Rudd Jones, 1950; Hellmers & Dowson, 1953). No consistent differences in pathogenicity to potatoes were observed among the bacteria considered in the present paper, but in certain biochemical activities they could be divided into three groups which were considered to represent the three species, *E. carotovora*, *E. atroseptica* and *E. aroideae*. Whether they should be regarded as separate species, or strains of one organism, is a matter of opinion.

Attempts to distinguish between the pectolytic *Erwinia* species have been based mainly on their fermentative abilities, and the present work has shown that in such studies different results can be obtained when different basal media and indicators are employed. The contradictory results of previous studies of maltose and glycerol fermentation may be explained in the light of this observation. It is suggested that where organic media have been used, the action of the bacteria on the basal media alone may have masked their effect on the carbohydrate under test. When the inorganic basal medium was used, acid produced in the utilization of extraneous C by *E. carotovora* and *E. aroideae* may have been recorded as acid produced in utilization of the known C source when brom thymol blue was the indicator.

The identification of *E. aroideae* of the present studies and *E. aroideae* of Holdeman & Burkholder (1956) as the same organism is open to question. Throughout the literature the cultural characteristic which has served to distinguish *E. aroideae* has been its inability to form gas in carbohydrate fermentation. This has been considered an unsatisfactory criterion for separating species, since some strains of *E. atroseptica* and *E. carotovora*, and even the same strain at different times of testing, have been observed to be anaerogenic. *E. aroideae* of Holdeman & Burkholder agrees with the original *E. aroideae* (Townsend, 1904) in its maximum temperature for growth (40° C.) which is higher than that of *E. aroideae* strains of the present study. It differs from the latter, however, in its inability to utilize maltose and ethyl alcohol.

From the extensive literature on the coliform soft-rot organisms, and the varied results which it contains, their taxonomy would appear to be difficult. However, the lines along which they, and indeed most plant pathogenic bacteria, have been studied have varied little. If they are to be more fully understood, it is necessary to study their biochemical activities further and to use other means of investigation—serology, bacteriophage, antibiotics, etc.—available to bacteriology.

This work was carried out as part of a programme of research at the Agricultural Research Council's Potato Storage Investigation, Sutton Bonington. My thanks are due to those who provided cultures, to the Advisory Officers in plant pathology through whom material for study was obtained, and to the Plant Pathology Department, Rothamsted Experimental Station, for preparing the antisera.

REFERENCES

APPEL, O. (1902). Der Erreger der 'Schwarzbeinigkeit', bei den Kartoffeln. *Ber. dtsch. bot. Ges.* **20**, 128–129.

AYERS, S. H., RUPP, P. & JOHNSON, W. T. (1919). A study of the alkali-forming bacteria in milk. *Bull. U.S. Dep. Agric.* no. 782.

BROWN, W. (1915). Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. *Ann. Bot., Lond.*, **29**, 313–348.

BURKHOLDER, W. H., & SMITH, W. L. (1949). *Erwinia atroseptica* (van Hall) Jennison and *Erwinia carotovora* (Jones) Holland. *Phytopathology*, **39**, 887–897.

DOWSON, W. J. (1940). Identity of the bacterium causing potato blackleg. *Nature, Lond.*, **114**, 263.

DOWSON, W. J. (1941). Soft rots due to green fluorescent bacteria. *Trans. Brit. mycol. Soc.* **25**, 215–216.

DOWSON, W. J. (1957). *Plant diseases due to bacteria.* Cambridge University Press.

ELROD, R. P. (1941). Serological studies in Erwineae. II. Soft-rot group, with some biochemical considerations. *Bot. Gaz.* **103**, 266–279.

VAN HALL, C. J. J. (1902). *Bijdragen tot de Kennis der Baklerieele Plantensiekten.* Inaug. Diss. Univ. Amsterdam. 198 pp.

HARDING, H. A. & MORSE, W. J. (1910). The bacterial soft rots of certain vegetables. Part 1. The mutual relationships of the causal organisms. *Bull. Vermont agric. Exp. Sta.* no. 147, 241–360.

HELLMERS, E. & DOWSON, W. J. (1953). Further investigations of potato blackleg. *Acta agric. Scand.* **3**, 103–112.

HOLDEMAN, Q. L. & BURKHOLDER, W. H. (1956). The identity of barn rots of flue-cured tobacco in South Carolina. *Phytopathology*, **46**, 69–72.

JONES, L. R. (1901). *Bacillus carotovorus* n.sp., die Ursache einer weichen Faulnis der Mohre. *Zbl. Bakt.* (Abt. II), **7**, 12–21, 61–68.

LACEY, M. S. (1926). Studies in Bacteriosis. XIII. A soft-rot of potato tubers due to *Bacillus carotovorus* and a comparison of the cultural, pathological and serological behaviour of various organisms causing soft-rot. *Ann. appl. Biol.* **13**, 10.

LEACH, J. G. (1930). The identity of the potato blackleg pathogene. *Phytopathology*, **20**, 743–751.

MASSEY, A. B. (1924). A study of *Bacillus aroideae* Townsend, the cause of soft-rot of tomato, and *B. carotovorus* Jones. *Phytopathology*, **14**, 460–477.

MINISTRY OF HEALTH (1939). *The bacteriological examination of water supplies.* H.M. Stationery Office, Report no. 71.

NOBLE, M. & GRAHAM, D. C. (1956). A selective medium for the isolation of coliform soft-rot bacteria from plant tissue. *Nature, Lond.*, **178**, 1479–1480.

NOBLE, M. & MARSHALL, M. (1952). A note on blackleg of potato. *Plant Pathology*, **1**, 134.

OXFORD, A. E. (1944). Production of a soluble pectinase in a simple medium by certain plant-pathogenic bacteria belonging to the genus *Pseudomonas*. *Nature, Lond.*, **154**, 271–272.

PATON, A. M. (1958). Pectin decomposing strains of *Pseudomonas*. *Nature, Lond.*, **181**, 61–62.

RUDD JONES, D. (1950). On the nomenclature and identity of the coliform soft-rot bacteria. *Trans. Brit. mycol. Soc.* **33**, 73–81.

SMITH, E. F. (1920). *An introduction to bacterial diseases of plants*, 688 pp. Philadelphia: W. B. Saunders Co.

STAPP, C. (1928). Die Schwarzbeinigkeit und Knollennassfaule der Kartoffel. *Arb. biol. Abt.* (*Anst.-Reichsanst.*), *Berl.*, **16**, 643–703.

TOWNSEND, C. O. (1904). A soft-rot of the Calla Lily. *Bull. U.S. Bur. Pl. Ind.* no. 60.

WALDEE, E. L. (1945). Comparative studies of some peritrichous phytopathogenic bacteria. *Iowa St. Coll. J. Sci.* **19**, 435–484.

WINSLOW, C. E. A., BROADHURST, J., BUCHANAN, R. E., KRUMWIEDE, C., ROGERS, L. A. & SMITH, G. H. (1920). The families and genera of the bacteria. Final report of the Committee of the Society of American Bacteriologists on Characterisation and Classification of Bacterial Types. *J. Bact.* **5**, 191.

WOLF, F. A. & FOSTER, A. E. (1921). Studies in the physiology of some plant pathogenic bacteria. *Bull. N.C. agric. Exp. Sta.* no. 20.

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PROCEEDINGS, 1958

MEETINGS

10 October 1958. Meeting held at Birkbeck College, London, W.C. 1, at 11.0 a.m. Dr Mary Glynne, Vice-President, in the Chair for the morning session, and Dr C. J. Hickman, Vice-President, in the Chair for the afternoon session.

D. C. Smith. Experimental work on the physiology of lichens.

A series of experiments on the absorption of a variety of substances from solution by *Peltigera polydactyla* and some other lichens has shown that these plants possess powers of active absorption which seem remarkably strong in view of their slow growth rates. The substances used in the experiments include sugars, asparagine, glutamine, aspartic and glutamic acids, and phosphate. The order of magnitude of absorption is illustrated by the fact that during a 24 hr. period, *P. polydactyla* can actively absorb 30% of its own dry weight of sugar and 10% of its own dry weight of asparagine.

Experiments with dissected thalli of *P. polydactyla* show that the algal region is the main site of absorption in the thallus. This region also has higher rates of respiration and a greater nitrogen content—it is not surprising that this should be the region of highest metabolic activity in the lichen since this is where contact between the symbionts occurs. The relatively thick medulla of fungal hyphae which underlies the algal layer has a greater water content and may serve as a water reservoir for the metabolically more active algal region.

Although *P. polydactyla* absorbs organic nitrogen compounds at a high rate, it utilizes them very slowly—as might be expected from its slow growth rate. This phenomenon might prove to be a feature of general importance in the ecology of lichens. The possession of strong powers of absorption from solution by lichens may represent an adaptation to living in habitats where the supply of dissolved nutrients is very poor. If lichens utilize absorbed nutrients at a slow rate then they may be able to use their strong powers of absorption to build up a reserve of nutrients during the occasional periods when nutrients are abundant in their habitats; they could then call upon these reserves during times of starvation.

J. Webster. Experiments with the spores of aquatic Hyphomycetes.

See *Ann. Bot., Lond.* (in preparation).

K. McDonnell. Pectic enzymes and the *Fusarium* wilt of tomatoes.

See *Nature, Lond.*, **182**, 1025–1026, 1958.

R. O. Sharples. The development of cankers due to *Gloeosporium perennans*, with a note on the occurrence of the perfect stage.

See *Rep. agric. hort. Res. Sta. Bristol*, 1957, p. 105, and *J. hort. Sci.* **34** (in the Press).

R. T. Burchill. The perennation of the apple mildew fungus *Podosphaera leucotricha* (Ell. & Ev.) Salm.

See *Rep. agric. hort. Res. Sta. Bristol*, 1957, pp. 114–123.

ANNUAL GENERAL MEETING

Saturday, 6 December 1958. The sixty-second Annual General Meeting was held in the rooms of the Linnean Society of London at 12.0 noon with the President, Dr F. B. Hora, in the Chair.

After the Minutes of the previous Annual General Meeting had been read and signed, the President recorded with regret the deaths during the year of Dr G. R. Bisby, Mr J. T. Duncan, Mr H. M. Parker and Mr E. W. Swanton. He then briefly reviewed the year's activities.

The Treasurer presented his Annual Statement which was accepted nem. con.

The following Officers and Council were elected for 1959: *President*: P. W. Brian; *Vice-Presidents*: G. C. Ainsworth, E. C. Large; *Treasurer*: W. Buddin; *Secretary*: J. G. Manners; *Foray Secretary*: Mrs S. M. Francis; *Plant Pathology Committee Secretary*: E. W. Buxton; *Editors*: S. D. Garrett and Miss G. M. Waterhouse; *Members of Council*: P. K. C. Austwick, P. A. Briggs, and W. J. Byford (retiring members: Miss J. Fraymouth, P. H. Gregory, T. J. Wallace); *Member of Foray Committee*: P. K. C. Austwick; *Members of Plant Pathology Committee*: P. R. Day and E. Evans.

The President, on behalf of Council, then moved that the proposed new Rules already circulated should replace the existing Rules of the Society. An amendment to the proposed new Rule dealing with the procedure for the election of Members of Council was then moved and carried, and it was therefore decided, on a motion from the Chair, to refer the proposed new Rules back to Council for further consideration.

At 2.30 p.m. the President gave an address entitled 'Quantitative experiments on Toadstool production in woods'. A vote of thanks to the President for his address was moved by Dr J. Ramsbottom, seconded by Mr D. A. Reid, and carried with acclamation.

REVIEW

Micro-Organisms in the Soil. By ALAN BURGES. (London: Hutchinson and Co. 1958. [Hutchinson University Library: Biological Sciences.]) Pp. 188, 6 text-figures. Price: 10*s*. 6*d*.

This book has been designed to give a general survey of the distribution of fungi, bacteria, actinomycetes, algae, protozoa and nematodes in the soil. The treatment is primarily an ecological one and an attempt is made to discuss the factors which govern micro-organisms in soil and their activity. Although all these groups of organisms are considered, as might be expected from one with Prof. Burges's interests, the major part of the book is in fact concerned with fungi in soil. For this group he has given us an interesting, lucid account of present knowledge, with some admirable comments on its limitations, that will commend itself to all interested in soil fungi. One feels, however, that perhaps the other organisms have not received quite such good treatment, though admittedly the book is a small one with probable limitations on size. Under such circumstances the problem of what to include and what to exclude is always difficult, but if organisms are mentioned in the book, either in tables or in text, they should be mentioned in the chapter on organisms present in soil. Amongst those neglected are the Acrasieae and other larger amoeboid organisms; while not a major group, they are not uncommon in soil and deserve mention. Most autotrophic bacteria also receive scant mention here. Other points that may be mentioned are that it should be pointed out that many soil bacteria are pleomorphic, a factor that largely nullifies the type of classification given on p. 31. To say that most amoebae 'are about 200–500 μ long', is to imply that soil forms are much larger than in fact they are, since most are about 10–40 μ long. It is also a pity that the recent *Bulletin on Laboratory Methods for Work with Plant and Soil Nematodes* (H.M. Stationery Office) is not mentioned.

The book is commendably free from errors and misprints and its low price should place it on the bookshelf of all interested in the life of micro-organisms in soil.

J. H. WARCUP

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Plant Pathology Committee: *List of Common British Plant Diseases*, 3rd Ed. 1945. Price net, 5*s*. Postage, 6*d*.

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A. A. Pearson & R. W. G. Dennis: *Revised list of British Agarics and Boleti. Ex* Vol. 31, Pts. 3 & 4. 1948. Price net, 5*s*. Postage, 5*d*.

J. Ramsbottom & F. L. Balfour-Browne: *List of Discomycetes recorded from the British Isles. Ex* Vol. 34, Pt. 1. 1951. Price net, 5*s*. Postage, 6*d*.

Fifty Year Index to The Transactions, Vols. 1–30; 1896–1946. Compiled by G. R. Bisby. Price net, 21*s*. Postage, 9*d*.

E. M. Blackwell: Haustoria of *Phytophthora infestans* and some other species. *Ex* Vol. 36, Pt. 2. 1953. Price 4*s*. Postage, 4*d*.

K. Sampson & J. H. Western: *Diseases of British Grasses and Herbage Legumes*. 2nd Edition. 1954. Price 15*s*. Postage, 1*s*. 1*d*.

Hilda M. Canter: *Annotated List of British Aquatic Chytrids. Ex* Vol. 36, No. 4. 1953. Price 3*s*. Postage, 4*d*.

Malcolm Wilson & G. R. Bisby: *List of British Uredinales. Ex* Vol. 37, No. 1. 1954. Price 3*s*. Postage, 4*d*.

Agnes H. S. Brown & George Smith. *The genus* Paecilomyces *Bainer and its perfect stage* Byssochlamys *Westling. Ex* Vol. 40, No. 1. 1957. Price 12*s*. 6*d*. Postage, 6*d*.

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A key to the genera of the agarics and Boleti. By A. A. Pearson. 32 pp. 1950. (Out of print. New edition in preparation.)

Guide to the literature for the identification of British fungi. 1952. Price 2*s*. 6*d*. Postage, 4*d*.

Census Catalogue of British Lichens. By W. Watson. 1953. Price 7*s*. 6*d*. Postage, 5*d*.

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INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and sub-headings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, The Royal Society, Burlington House, London, W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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